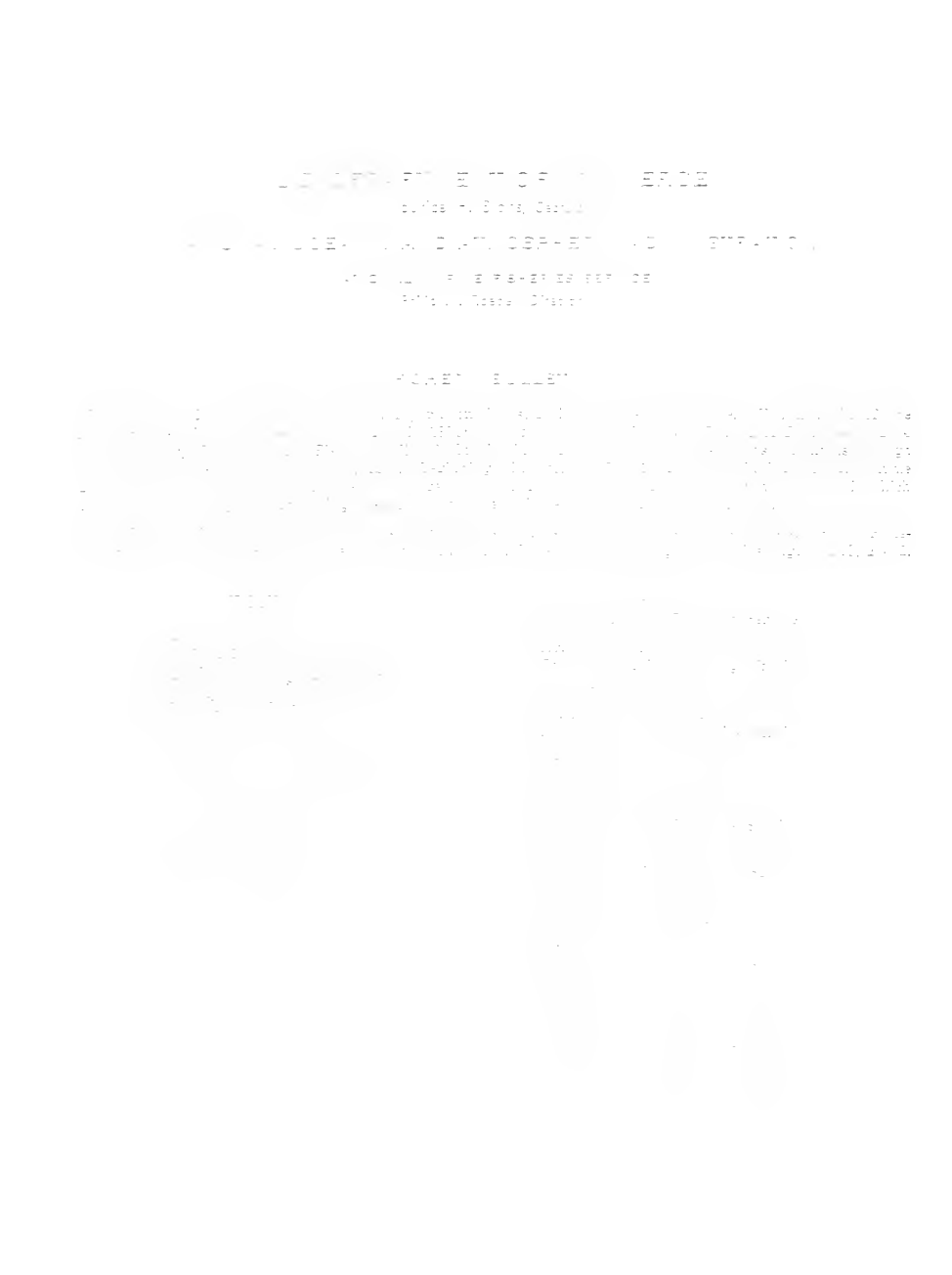

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IN MEMORIAM

Wilbert McLeod Chapman and Milner Baily Schaefer

Fisheries science in particular and society in general suffered two tragic losses within the period of only a month with the deaths of W. M. Chapman on June 25, 1970, and M. B. Schaefer on July 26, 1970. It is indeed a strange and sad commentary that these two men whose careers were intimately entwined since college days should pass within such a short time of each other.

Death is never easy to accept; it is particularly hard to do so when it occurs at an untimely age. Both of these brilliant men, we would have hoped, would have been with us for years to come. Both were unique, each in his own way, and while the world adjusts to such events, each is in a very real sense irreplaceable.

I had the opportunity to work rather closely with both of them in California over most of the past two decades. Wib Chapman was a member of the California Marine Research Committee for many years, during most of which I served as that body's secretary. It was a challenge to try to capture the essence of his remarks. The breadth of his knowledge and the incisiveness of his thinking stimulated all of us to higher goals, and we who were close to him are better today for the good fortune of his friendship.

"Benny" Schaefer was equally brilliant. His expositions on the scientific method and population dynamics before the Inter-American Tropical Tuna Commission were models of translation into lay terms of highly complex mathematical theories applied to living resources. Again a personal note. A few years ago Benny was a consultant to the California Department of Fish and Game during the formation of that body's Fish and Wildlife Plan and we worked closely in developing the philosophy behind the sections concerned with living marine resources. His imprint is deeply ingrained in that document and in subsequent legislation, as well as in my thinking.

And, as Wib Chapman had a large input in that task, so did Benny into the deliberations of the Marine Research Committee. Meantime both worked diligently as members of the California Marine Advisory Committee on Marine and Coastal Affairs. While these men will rightfully be remembered for their major contributions to national and international affairs, their energy and interests were such that they encompassed an amazingly broad spectrum. Each of their contributions to the State of California is more than most men could accomplish in a lifetime devoted to that pursuit alone. One hears parallel stories throughout the scientific and fisheries communities.

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Wilbert McLeod Chapman was born in Kalama, Washington, on March 31, 1910. He died in San Diego, California, on June 25, 1970, and is survived by his wife of 35 years, Mary Elizabeth, and five of their six children.

He did both his undergraduate and graduate work at the University of Washington, obtaining his Ph.D. (fisheries) in 1937. His publications, ranging from morphology and systematic ichthyology through fisheries economics and international law, number some 250. One of these, *Fishing in Troubled Waters*, is a book recounting his experiences as a fisheries development officer in the South Pacific during World War II. It is fascinating reading and makes one regret all the more that the other books he had in mind will never be forthcoming. He was particularly proud of his papers on systematics and morphology and always spoke fondly of that part of his career.

His honors were many: among them he was a Fellow of the Guggenheim Foundation and of the California Academy of Sciences, Man of the Year of the National Fisheries Institute in 1966,

and the recipient of the First Sea Grant College Award in 1968.

He began his professional career in 1933 with the International Fisheries (now Halibut) Commission. He was later employed by the Washington State Department of Fisheries, the U.S. Fish and Wildlife Service, and, in 1943, by the California Academy of Sciences where he was Curator of Fishes until 1947. It was during this period that he served in a civilian capacity in the South Pacific, his job being to develop subsistence fisheries at advanced island bases.

In 1947, Dr. Chapman became director of the School of Fisheries at the University of Washington. He left there in 1948 to become the first Special Assistant to the Under Secretary of State for Fish and Wildlife. In 1951 he became Director of Research for the American Tunaboat Association; a decade later he joined the Van Camp Sea Food Company as Director of the Division of Resources. When Van Camp was acquired by the Ralston Purina Company in 1968, he became Director, Marine Resources, of that firm, a position he held until his death.

★ ★ ★

Milner Baily Schaefer was born in Cheyenne, Wyoming, on December 14, 1912. He died in San Diego, California, on July 26, 1970. He is survived by his wife, Isabella, and three children.

Dr. Schaefer obtained his B.S. degree cum laude from the University of Washington in 1936 and his doctorate from the same institution in 1950. He worked for the Washington State Department of Fisheries from 1935 to 1938 and for the International Pacific Salmon Fisheries Commission from 1938 until 1942.

Following wartime duty with the Navy, he joined the U.S. Fish and Wildlife Service in 1946, serving first as a fishery research biologist in the South Pacific Fisheries Investigations at Stanford, and from 1948 to 1950 as Chief, Research & Development, Pacific Oceanic Fishery Investigations in Honolulu.

He became Director of Investigations of the Inter-American Tropical Tuna Commission in 1951, holding that post until he became Director of the Institute of Marine Resources and Professor of Oceanography, Scripps Institution of Oceanography, University of California, in 1962. He remained there until his death save for an 18-month period in 1967-69 during which he was Science Adviser to Secretary of the Interior Stewart Udall.

Among other honors, he was a fellow of the California Academy of Sciences and a member of the National Academy of Sciences. He wrote more than 100 scientific papers, particularly in the area of population dynamics and fisheries development and utilization. He served on a multitude of panels at the international, national and state levels. Despite his huge workload, he always found time to discuss individual problems with people both large and small, and to administer and develop first the Inter-American Tropical Tuna Commission and later the Institute of Marine Resources in an exemplary manner, setting standards for each that others will be hard-pressed to equal.

★ ★ ★

This recitation cannot give a measure of these men: their unflagging energy, their knowledge in fields far apart from fisheries, their ability as raconteurs, their good fellowship. Nor does it give a measure of their contributions to the nation and to the world, contributions that will help make it a better place in which to live for a long time to come.

Philip M. Roedel

KINDS AND ABUNDANCE OF FISH LARVAE IN THE EASTERN TROPICAL PACIFIC, BASED ON COLLECTIONS MADE ON EASTROPAC I

ELBERT H. AHLSTROM[1]

ABSTRACT

This paper deals with kinds and counts of fish larvae obtained in 482 oblique plankton hauls taken over an extensive area of the eastern tropical Pacific on EASTROPAC I, a four-vessel cooperative survey made during February-March 1967. On the basis of abundance of larvae, the dominant fish group in oceanic waters are the myctophid lanternfishes (47 %), gonostomatid lightfishes (23 %), hatchetfishes, Sternoptychidae (6 %), bathylagid smelts (5 %). Scombrid larvae ranked fifth, and exceeded 2 % of the count.

Two kinds of larvae were outstandingly abundant: larvae of the lanternfish *Diogenichthys laternatus* made up over 25 % of the total, while larvae of the gonostomatid genus *Vinciguerria* made up almost 20 %. More fish larvae were obtained per haul, on the average, in the eastern tropical Pacific than were obtained per haul in the intensively surveyed waters of the California Current region off California and Baja California.

EASTROPAC I was the first and most wide-ranging of a series of cooperative cruises made in the eastern tropical Pacific between February 1967 and April 1968. A vast expanse of the eastern tropical Pacific was surveyed on EASTROPAC I, extending from lat 20° N to 20° S, and from the American coasts offshore to long 126° W (Fig. 1). Four research vessels participated in EASTROPAC I: *Alaminos* operated by Texas A & M, occupied the inner pattern, while *Rockaway* operated by the U.S. Coast Guard, *David Starr Jordan* operated by the Bureau of Commercial Fisheries (now the National Marine Fisheries Service), and *Argo* operated by the Scripps Institution of Oceanography, occupied patterns successively seaward. The oceanographic, biological, and meteorological data collected on EASTROPAC cruises will be graphically presented in a series of EASTROPAC atlases, including generalized charts dealing with fish eggs and larvae.

The present paper is the result of a chain of events that began 2 decades ago, at the initiation of CalCOFI (California Cooperative Oceanic Fisheries Investigations) in which a large-scale sea program was set up to investigate the distribution and abundance of sardine spawning, and the factors underlying fluctuations in survival of the early life-history stages of sardines. The plankton collections not only contained eggs and larvae of sardine but those of most other pelagic fishes in the California Current region. A decision was made to attempt to identify and enumerate all fish larvae in the collections in order to obtain more precise information about the ecological associates of the sardine. At that time few fish larvae, other than those of the sardine and anchovy, could be identified.

Within a few years most kinds of fish larvae were identified to genus or species. Once the larvae were identified and enumerated, it became obvious that this was an exceptionally useful tool for evaluating fish resources. Most oceanic fishes have pelagic eggs and/or larvae that are distributed in or just below the photic zone, i.e. within the upper 150 to 200 m of depth. At no other time in their life histories are so many kinds of fishes associated together—deep-sea fishes (mesopelagic and bathypelagic) as well as epipelagic species—where they can be collected quantitatively with a single type of gear, a plankton net.

Once the larvae of the pelagic fish fauna of a region, such as those in the California Current region, are known, there is a large trans-

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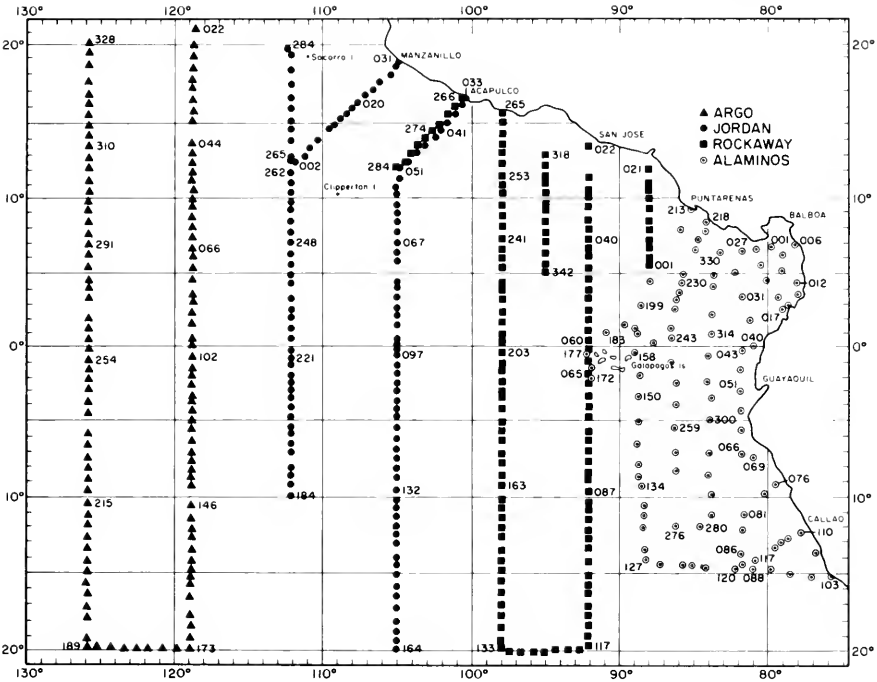

FIGURE 1.—Location of plankton stations occupied by four research vessels participating in EASTROPAC I. Symbols for vessels indicated in legend above. Samples collected from *Argo* are numbered as 11.000 series (as 11.022, 11.173), samples from *David Starr Jordan* as 12.000 series, *Rockaway* samples as 13.000 series and *Alaminos* samples as 14.000 series.

ference of the accumulated knowledge and skills for work in other areas, such as, in this instance, the eastern tropical Pacific. My study was undertaken to demonstrate the value of identifying all elements of the fish fauna of tropical regions, rather than restricting interest to scombrid larvae. Much information can be gained for little extra expense (a few percent of the cost of collecting the material at sea). Of equal consequence, identification of all kinds of fish larvae can be made more critically including scombrid larvae.

METHODS OF MAKING ZOOPLANKTON COLLECTIONS

Three nets, differing in size and in coarseness of mesh, were employed to collect zooplankton and micronekton on EASTROPAC cruises. In this paper I am concerned primarily with oblique hauls made with the net of intermediate size and mesh—a net, 1-m mouth diameter, constructed of 505 μ nylon (Nitex) cloth, with approximately a 5 to 1 ratio of effective straining surface (pore area) to mouth area. This net was paired in an assembly frame with a finer-

meshed net when hauled obliquely, but was used alone for taking surface hauls. The finer-meshed net was 0.5 m in diameter at the mouth, constructed of 333 μ Nitex cloth, with approximately an 8 to 1 ratio of effective straining surface to mouth area. The third net, used for collecting micronekton, had a 5-ft square mouth opening and was constructed of mesh measuring approximately 5.5 $\times$ 2.5 mm; this net could not be operated from the research vessel *Rockaway* on EASTROPAC I but was employed from the other three vessels.

Usually four zooplankton collections were made at each "biological" station: an oblique collection and a surface collection with the 1-m net, an oblique collection with the 0.5-m net, and an oblique collection with the micronekton net.

In taking oblique plankton hauls, the 1-m net was paired in an assembly frame with the 0.5 m net. The assembly of nets was fastened to the towing cable by a bridle about 5 m above a 100-lb weight. The assembly was lowered to depth by paying out 300 m of towing cable at the controlled rate of 50 m of wire per minute. The assembly remained at depth for 0.5 min and then was retrieved at a uniform rate of 20 m per min. Total towing time was about 21.5 min. Towing speed was ca. 2 knots. The depth reached by the net was estimated from the angle of stray (departure from the vertical) of the towing cable. We sought to maintain an angle of stray of 45°, which lowered the assembly to a depth of approximately 210 m. Our concern was to sample the upper 200-m stratum. The average depths of hauls taken by the four research vessels are summarized in Table 1. Over 80 % of the hauls made on EASTROPAC I were lowered to depths of 200 m or more, and nearly 95 % reached depths of 180 m or greater. However, two hauls were exceptionally shallow (71-90 m), and nine additional hauls were taken to depths of less than 150 m.

Usually four paired net-assembly hauls were taken per day, spaced at about 6-hr intervals. Although the four hauls were planned to be taken at about midnight, dawn, noon, and sunset, the timing of hauls was not coordinated between research vessels. The middle-of-the-night hauls

TABLE 1.—Depth of paired oblique plankton hauls taken by the four research vessels on EASTROPAC I. (Net lowered by paying out 300 m of towing cable)

Average depth of haul	Number of hauls taken to each depth interval from				
	Argo	*David Starr Jordan*	*Rockaway*	*Alaminos*	All vessels
M					
70.1- 80.0	--	--	--	1	1
80.1- 90.0	--	--	--	1	1
90.1-100.0	--	--	--	--	--
100.1-110.0	--	--	--	1	1
110.1-120.0	--	--	--	--	--
120.1-130.0	2	--	--	3	5
130.1-140.0	1	--	--	1	2
140.1-150.0	--	1	--	--	1
150.1-160.0	--	--	1	2	3
160.1-170.0	2	--	2	2	6
170.1-180.0	2	2	2	1	7
180.1-190.0	15	5	4	5	29
190.1-200.0	21	10	11	10	52
200.1-210.0	41	59	58	30	188
210.1-220.0	26	44	57	41	168
220.1-230.0	9	--	3	5	17
230.1-240.0	--	--	1	--	1
Total	119	121	139	103	482

were all taken before midnight (2201-2400) on *Rockaway*, for example, while on *Argo* most hauls were made after midnight (between 0001 and 0400 hr). The time of day of occupancy of stations (based on the midtime of each haul) is summarized by hourly intervals in Table 2. At least some hauls were taken during every hour of the day, although fewer than 10 (2-8) were obtained during six of the hourly intervals. Fewest hauls were obtained between 0901 and 1000 hr (2 hauls) and between 2101 and 2200 hr (4 hauls), whereas the largest number of hauls were taken between 2201 and 2300 hr (59 hauls) and between 1001 and 1100 hr (53 hauls). Hauls were made with equal frequency during the four periods of the day on *Argo*, *Jordan*, and *Rockaway;* most plankton hauls were taken near midnight or noon from *Alaminos*.

The numbering system for observations employed on EASTROPAC cruises made use of five digits divided into two groups, as 11.022, 12.002, etc. The outer digit preceding the period is the cruise number common to all vessels participating in a given EASTROPAC cruise; for EASTROPAC I, this number is 1. The other digit preceding the period is the identifying number given to each research vessel, with the lowest

TABLE 2.—Hour of day that paired oblique plankton hauls were taken from the four research vessels participating in EASTROPAC I. (Midtime of haul used.)

Hours of day	Number of hauls taken during each hour of the day from				
	Argo	*David Starr Jordan*	*Rockaway*	*Alaminos*	All vessels
0001-0100	7	10	0	3	20
0101-0200	8	7	0	2	17
0201-0300	5	2	0	0	7
0301-0400	9	0	7	0	16
0401-0500	1	1	17	1	20
0501-0600	2	9	10	3	24
0601-0700	7	10	1	1	19
0701-0800	13	10	0	0	23
0801-0900	7	0	0	0	7
0901-1000	0	0	0	2	2
1001-1100	1	0	26	26	53
1101-1200	1	5	5	10	21
1201-1300	7	22	3	1	33
1301-1400	12	3	1	4	20
1401-1500	8	0	0	0	8
1501-1600	1	1	12	1	15
1601-1700	0	0	10	3	13
1701-1800	8	6	12	6	32
1801-1900	7	19	1	0	27
1901-2000	10	1	0	0	11
2001-2100	3	3	0	0	6
2101-2200	0	1	0	3	4
2201-2300	2	2	23	32	59
2301-2400	0	9	11	5	25
Total	119	121	139	103	482

number given to the offshore vessel. The three digits following the period are numbers given to observations made from each vessel during a cruise, numbered sequentially. Not all "stations" included oblique plankton hauls; hence there are gaps in numbers applied to plankton collections.

The locations of plankton stations occupied by the four research vessels participating in EASTROPAC I are shown in Figure 1. Samples collected from the *Argo* are designated as the 11.000 series, samples from the *David Starr Jordan* as 12.000 series, *Rockaway* samples as 13.000 series and *Alaminos* samples as 14.000 series. In tables to follow, the series of samples taken by each vessel is designated by the above identifying series numbers. The aggregate of stations occupied by each vessel is referred to in text discussions as its pattern.

PROCESSING SAMPLES ASHORE

As noted above, only samples from 1-m oblique net hauls were sorted routinely for fish eggs and larvae. As a rule the entire sample was sorted; in fact only six collections out of 482 were aliquoted — four collections were split into 50 % aliquots, two collections into 25 % aliquots.

The author made all identifications and counts of larvae from EASTROPAC I collections. Actual counts of larvae rather than standardized values (see below) are used in tabulation throughout this paper, except one (Table 7). There are several reasons why I chose to do this. As indicated previously, all hauls were made in a roughly comparable fashion. In many studies the investigator is interested in the presence or absence of the larvae of a given species or assemblage of species as such relate to water masses, community composition, time of day, etc. Such information is most readily obtained from records of actual counts. Some statistical tests require the use of original counts rather than standardized data. For persons interested in deriving standardized counts comparable with those employed for CalCOFI data (Ahlstrom, 1953), standard haul factors for the 482 oblique hauls taken with the 1-m net on EASTROPAC I are given in Appendix Table 7.

Two major considerations in the quantitative sampling of fish larvae for resources evaluation are (1) how well has their depth range been covered and (2) how effectively have the larvae been sampled within this layer?

We do not have direct answers to either of these questions from EASTROPAC cruises. No studies were made on depth distributions of fish eggs and larvae in the EASTROPAC area. As will appear, fewer fish larvae were obtained during daylight hours than in night hauls; however, we lack information on how completely larvae were sampled in night hauls.

DEPTH DISTRIBUTION OF FISH LARVAE

Although collecting methods used on EASTROPAC did not permit a study of depth distribution of fish larvae, such information for the California Current region off California and Baja California and in a less detailed way for the NORPAC Expedition of 1955 are available (Ahlstrom, 1959).

In the California Current region, most fish eggs and larvae were distributed within the up-

per mixed layer or in the upper portion of the thermocline, between the surface and approximately 125 m. Of the 15 most common kinds of fish larvae taken in vertical distribution series, 12 were so distributed (ibid., p. 134). Two of the kinds that occurred most commonly below the thermocline were bathylagid smelts, closely related to the two common bathylagid smelts taken on EASTROPAC I.

On the NORPAC Expedition of August 1955, two depth strata were sampled at most stations; a closing net, fastened to the towing cable 200 m below a standard open plankton net, sampled the level between 262 and 131 m on the average, while the upper net sampled from the surface to approximately 131 m deep. Only about one-ninth as many larvae were taken in the closing net hauls as in the upper net hauls; fully half of these were larvae of hatchetfish, family Sternoptychidae, largely absent from upper net hauls. The two most abundant kinds of fish larvae taken on EASTROPAC I were those of the myctophid lanternfish, *Diogenichthys laternatus*, and of the gonostomatid lightfish, *Vinciguerria* spp. In NORPAC collections, only 3 % of the larvae of *D. laternatus* were taken in the closing net hauls and only 2 % of the *Vinciguerria* larvae. Among the kinds of larvae common to both the NORPAC and EASTROPAC surveys that occurred in significant numbers in the deeper NORPAC collections were those of *Chauliodus* (72 % taken in closing net hauls), *Protomyctophum* (48 %) and *Idiacanthus* (32 %).

Inasmuch as the vertical distribution studies in the California Current region had pointed up the importance of the thermocline in the depth distribution of larvae, the pattern of thermocline depth was analyzed for EASTROPAC I (Table 3).

Thermocline depth was invariably shallow in the inner pattern occupied by *Alaminos* (data not included in Table 3); the greatest depth recorded was only 40 m, and the majority of observations were at depths shallower than 20 m. Along the six station lines covered in Table 3, thermocline depths were shallowest near the equator, and usually were deepest at the northern (20-15° N) and southern (15-20° S) ends of the lines. The thermocline also deepened offshore; approximately three-fourths of the records of thermocline depths of 50 m or greater were from the two outer lines, occupied by *Argo*.

Most oblique plankton hauls taken on EASTROPAC I sampled to depths of 200 m or more (Table 2), hence sampled considerably deeper than the thermocline in all parts of the EASTROPAC area.

EFFECTIVENESS OF SAMPLING FISH LARVAE IN DAYLIGHT HAULS AS COMPARED WITH NIGHT HAULS

Fewer fish larvae were obtained in hauls made during daylight hours than at night (Table 4). Original (unstandardized) counts of larvae averaged 2.76 times as many in night hauls as in day hauls, 285 larvae per occupancy as compared with 103 larvae. Hauls made within 1 hr of sunrise or sunset contained intermediate numbers of larvae, averaging 217 larvae per occupancy.

TABLE 3.—Summary of records of thermocline depths along six station lines occupied by the research vessels *Rockaway*, *David Starr Jordan*, and *Argo* on EASTROPAC I.

Station line along longitude	Range in depth of thermocline (*m*) at latitudes								All latitudes
	20-15° N	15-10° N	10-5° N	5° N-0°	0-5° S	5-10° S	10-15° S	15-20° S	
92° W	--	0-15	7-14	5-29	0-16	15-40	24-45	30-54	0-54
98°	16-30	13-68	23-44	5-13	2-27	13-32	20-48	40-60	2-68
105°	--	37-50	27-44	0-20	0-28	23-45	26-55	54-66	0-66
112°	8-42	41-79	32-58	0-37	2-22	33-52	--	--	0-79
119°	36-67	44-90	42-55	0-85	0-65	34-76	50-73	30-71	0-90
126°	52-116	45-79	35-49	0-42	0-60	40-71	43-71	43-70	0-116
% abs. with T. D. shallower than 10.1 m	17 %	8 %	7 %	46 %	43 %	0	0	0	20 %
% abs. with T. D. deeper than 49.9 m	56 %	46 %	9 %	11 %	9 %	25 %	35 %	63 %	26 %

TABLE 4.—Occurrence (positive hauls) and abundance (original counts) of fish larvae in day hauls as compared with night hauls and with hauls taken within 1 hr of sunrise or sunset, summarized by family and vessel pattern.

Vessel pattern and family	Day hauls			Night hauls				Hauls within ± 1 hr of sunrise or sunset			Total hauls		
	Number positive hauls	Total larvae	Average number per occupancy ($\bar{D}$)	Number positive hauls	Total larvae	Average number per occupancy ($\bar{N}$)	$\bar{N}/\bar{D}$	Number positive hauls	Total larvae	Average number per occupancy	Number positive hauls	Total larvae	Average number per occupancy
11.000 series													
Arca	(44)[1]			(42)				(33)			(119)		
Myctophidae	43	1,129	25.7	42	4,412	105.0	4.09	33	1,452	44.0	118	6,993	58.8
Gonostomatidae	44	727	16.5	42	2,619	62.4	3.78	32	1,317	39.9	118	4,663	39.2
Sternoptychidae	36	327	7.4	33	343	8.2	1.10	21	253	7.7	90	923	7.8
Bathylagidae	15	96	2.2	21	103	2.4	1.12	13	85	2.6	49	284	2.4
Melamphaidae	26	65	1.5	23	65	1.6	1.05	17	35	1.1	66	165	1.4
Scombridae	5	21	0.5	13	29	0.7	1.44	8	187	5.7	26	237	2.0
All others	42	687	15.6	42	1,012	24.1	1.54	32	555	16.8	116	2,254	18.9
Total	44	3,052	69.4	42	8,583	204.4	2.95	33	3,884	117.8	119	15,519	130.5
12.000 series													
Jordan	(34)			(37)				(50)			(121)		
Myctophidae	34	1,257	37.0	37	5,389	145.6	3.94	50	4,628	92.6	121	11,274	93.2
Gonostomatidae	32	451	13.3	37	2,417	65.3	4.91	49	1,914	38.1	118	4,782	39.5
Sternoptychidae	22	452	13.3	21	503	13.6	1.02	31	757	15.1	74	1,712	14.1
Bathylagidae	20	105	3.1	20	168	4.5	1.45	33	223	4.5	73	496	4.1
Melamphaidae	21	43	1.3	28	58	1.6	1.25	28	70	1.4	77	171	1.4
Scombridae	9	80	2.4	16	129	3.5	1.49	19	133	2.7	44	342	2.8
All others	34	766	22.5	37	2,009	56.8	2.52	49	1,244	24.9	120	4,109	34.0
Total	34	3,154	92.9	37	10,763	290.9	3.13	50	8,969	179.5	121	22,886	189.1
13.000 series													
Rockaway	(65)			(59)				(15)			(139)		
Myctophidae	65	3,761	57.9	58	8,557	145.0	2.50	14	2,911	194.1	137	15,229	109.6
Gonostomatidae	62	1,138	17.5	59	5,608	95.0	5.43	14	1,383	92.2	135	8,129	58.5
Sternoptychidae	47	798	12.3	45	829	14.1	1.14	14	534	35.6	106	2,161	15.5
Bathylagidae	43	475	7.3	37	411	7.0	0.95	13	189	12.6	93	1,075	7.7
Melamphaidae	42	130	2.0	43	132	2.2	1.12	11	59	3.9	96	321	2.3
Scombridae	30	131	2.0	29	435	7.4	3.65	10	39	2.6	69	605	4.4
All others	62	1,009	15.5	59	2,422	41.1	2.65	15	429	28.6	136	3,860	27.8
Total	65	7,442	114.5	59	18,394	311.8	2.72	15	5,544	369.6	139	31,380	225.8
14.000 series													
Alaminos	(50)			(46)				(7)			(103)		
Myctophidae	46	2,523	50.5	43	6,640	144.3	2.86	7	2,254	322.0	96	11,417	110.8
Gonostomatidae	41	874	17.5	41	2,550	55.4	3.17	6	1,048	149.7	88	4,472	43.4
Sternoptychidae	31	312	6.2	31	565	12.3	1.97	5	14	2.0	67	891	8.7
Bathylagidae	40	1,015	20.3	43	1,809	39.3	1.94	6	201	28.7	89	3,025	29.4
Melamphaidae	28	69	1.4	26	104	2.3	1.64	5	27	3.9	59	200	1.9
Scombridae	16	65	1.3	25	448	9.7	7.49	5	222	31.7	46	735	7.2
All others	45	1,406	28.1	45	2,557	55.6	1.98	6	621	88.7	96	4,584	44.5
Total	46	6,264	125.3	46	14,673	318.9	2.55	7	4,387	626.7	99	25,324	245.9
Complete EASTROPAC 1	(193)			(184)				(105)			(482)		
Myctophidae	188	8,670	44.9	180	24,998	135.9	3.03	104	11,245	107.1	472	44,913	93.2
Gonostomatidae	179	3,190	16.5	179	13,194	71.7	4.35	101	5,662	53.9	459	22,046	45.7
Sternoptychidae	136	1,889	9.8	140	2,240	12.2	1.24	71	1,558	14.8	337	5,687	11.8
Bathylagidae	118	1,691	8.8	121	2,491	13.5	1.54	65	698	6.7	304	4,880	10.1
Melamphaidae	117	307	1.6	120	359	1.9	1.23	61	191	1.8	298	857	1.8
Scombridae	60	297	1.5	83	1,041	5.7	3.68	42	581	5.5	185	1,919	4.0
All others	183	3,868	20.0	183	8,090	44.0	2.20	102	2,849	27.2	468	14,807	30.7
Total	189	19,912	103.1	184	52,413	284.9	2.76	105	22,784	217.0	478	95,109	197.3

[1] Total number of hauls.

Larvae of some families of fishes were sampled almost as well in day hauls as in night hauls —including Sternoptychidae, Bathylagidae, and Melamphaidae. In contrast, less than one-fourth as many gonostomatid larvae and one-third as many myctophid larvae were taken in day hauls, on the average, as in night hauls. Catches of scombrid larvae were more variable with regard to time of sampling—the night-day ratio in the outer half of the EASTROPAC area was only about 1.5 to 1, whereas the ratio jumped to about 7.5 to 1 in the inner pattern occupied by *Alaminos*. Larvae collected about in equal amounts in day and night hauls were those known to occur principally below the thermocline.

Despite the lower abundance of larvae in day hauls as compared with night hauls, the percentage of hauls containing larvae of most families was only slightly lower (Table 5). The most marked day/night difference in frequency of occurrence was for scombrid larvae, these were taken in 45 % of night hauls, but in only 31 % of day hauls. In the discussions that follow I make use of all collection data, irrespective of time of collection.

TABLE 5.—Percentage of hauls containing larvae of the more abundant fish families on EASTROPAC I, grouped by day, night and dawn or sunset.

Family	Day hauls	Night hauls	Dawn or sunset hauls (± 1 hr)	All hauls
	%	%	%	%
Myctophidae	97.4	97.8	99.0	97.9
Gonostomatidae	92.7	97.3	95.2	95.0
Sternoptychidae	70.5	76.1	67.6	69.9
Bathylagidae	61.1	65.2	61.9	62.9
Melamphaidae	60.6	65.2	58.1	61.8
Scombridae	31.1	45.1	40.0	38.4
All others	94.8	99.5	97.1	97.1
Total	97.9	100.0	100.0	99.2

NUMBERS OF FISH LARVAE OBTAINED ON EASTROPAC I

Fish larvae were obtained in 478 of 482 oblique plankton tows made with the 1-m plankton net on EASTROPAC I. The number of larvae per collection ranged from 0 to 2,197, averaging 197 larvae (actual counts).

Differences in abundance of larvae with latitude are summarized for the four series in Table 6. Fish larvae were obtained in largest numbers, on the average, in an equatorial band extending from about lat 10° N to 5° S. The least productive waters for fish larvae were in the central water mass of the South Pacific, especially between lat 15° and 20° S.

Abundance of fish larvae also decreased offshore, averaging only 130 larvae per haul in the outer pattern, occupied by *Argo*, as compared with 246 larvae per haul in the inner pattern, occupied by *Alaminos*.

Tropical waters and oceanic waters are usually considered to be relatively unproductive, compared with temperate coastal regions such as the California Current region (Ryther, 1969). Hence, it is surprising to find that the average number of fish larvae obtained per haul on EASTROPAC I was larger than either on the CalCOFI cruises from the California Current region (Ahlstrom, 1969) or on NORPAC (un-

TABLE 6.—Total catches of fish larvae (actual counts) taken by the four research vessels on EASTROPAC I, summarized by latitude.

Latitude	*Argo* 11.000 Series		*David Starr Jordan* 12.000 Series		*Rockaway* 13.000 Series		*Alaminos* 14.000 Series		Total EASTROPAC I		
	No. hauls	No. larvae	No. hauls	No. larvae	No. hauls	No. larvae	No. hauls	No. larvae	No. hauls	No. larvae	Average no. larvae per haul
20° N-15° N	16	1,070	20	4,128	5	462	--	--	41	5,660	138.0
15° N-10° N	14	1,372	23	3,130	26	5,508	--	--	63	10,010	158.0
10° N- 5° N	14	2,516	14	3,344	29	10,104	15	5,167	72	21,131	293.5
5° N- 0°	14	4,797	15	4,403	14	4,331	27	11,329	70	24,860	355.1
0° - 5° S	14	2,089	18	5,454	14	4,350	17	5,042	63	16,935	268.8
5° S-10° S	13	1,370	15	1,051	14	2,360	16	2,113	58	6,894	118.9
10° S-15° S	14	1,512	8	863	15	2,337	28	1,673	65	6,385	98.2
15° S-20° S	20	793	8	513	22	1,928	--	--	50	3,234	64.7
Total	119	15,519	121	22,886	139	31,380	103	25,324	482	95,109	197.3

published data). Standard haul totals of larvae are used in this comparison (Table 7) not original counts. CalCOFI cruises repeatedly surveyed a coastal area extending 200 to 300 miles offshore between San Francisco, California, and Magdalena Bay, Baja California. NORPAC was the first comprehensive survey of the North Pacific, made in August-September 1955; the area surveyed by four CalCOFI vessels participating in NORPAC was between lat 20° and 45° N and offshore to long 150° W.

TABLE 7.—Comparison of the average number of fish larvae obtained per haul (standard haul values) EASTROPAC I, NORPAC, and CalCOFI cruises.

Cruises	Year	Number hauls	Average depth of hauls	Total number of fish larvae[1]	Average number larvae/haul
EASTROPAC I	1967	482	ca. 200 m	274,131	569
NORPAC	1955	196	ca. 260 m	27,000	[2]138
CalCOFI cruises	1956	1,407	ca. 140 m	408,140	290
	1957	1,493	ca. 140 m	493,550	331
	1958	1,852	ca. 140 m	456,020	246
	1959	2,182	ca. 140 m	470,450	216
	1960	1,826	ca. 140 m	504,980	277

[1] Standard haul totals.
[2] Data from two net hauls combined: an average of 124 larvae per haul were taken in upper net hauls (0 to 130 m) and an average of 14 larvae per haul in closing net hauls, sampling between ca. 260 and 130 m.

EASTROPAC hauls sampled a somewhat deeper stratum than hauls made on CalCOFI cruises, ca. 200 m as compared to ca. 140 m. As indicated previously, information is available for the majority of NORPAC stations on the relative abundance of fish larvae in the level between ca. 130 and 260 m (closing net hauls) as compared with the level above, 0 to 130 m. Only about one-ninth as many larvae were taken in the deeper hauls.

The difference between catches of larvae on EASTROPAC I and NORPAC are particularly marked—four times as many larvae were taken per haul, on the average, on EASTROPAC I as on NORPAC (both nets combined). For comparison with shallower CalCOFI hauls, I am assuming that 10 % of the EASTROPAC larvae were obtained in the level between ca. 140 and 200 m. The adjusted value for EASTROPAC larvae, 512 larvae per haul, on the average, is 1.55 times as large as the highest CalCOFI value listed (331 larvae per haul in 1957) and 2.35 times as large as the lowest value (216 larvae per haul in 1959).

The majority of EASTROPAC larvae were those of fishes which never attain a large size as adults—myctophids, gonostomatids, sternoptychids, etc.—hence numbers of larvae, per se, cannot be considered reliable indices of biomass. The familial composition of larvae was not dissimilar on NORPAC and EASTROPAC, however; hence this comparison of relative abundance of larvae is more relevant, as regards biomass, than the comparison with CalCOFI fauna.

KINDS OF FISH LARVAE OBTAINED ON EASTROPAC I

The kinds of larvae obtained on EASTROPAC I are summarized by family and vessel pattern in Table 8, the principal summary table in this paper. Larvae of more than 50 families are listed, but larvae of 10 families contributed 90 % of the total. The myctophids were the dominant group with 47.2 % of the larvae occurring in nearly 98 % of the collections. Gonostomatid larvae were about half as numerous, contributing 23.2 % of the larvae while occurring in 95 % of the collections. Hatchetfish larvae (Sternoptychidae) ranked third in abundance with 6 % of the larvae taken in 70 % of the hauls. Bathylagid larvae also exceeded 5 % of the total and occurred in 63 % of the collections. Scombrid larvae ranked fifth and exceeded 2 % of the count, followed by Bregmacerotidae, 1.9 %, Paralepididae, 1.7 %, Idiacanthidae, 1.0 %, Nomeidae, 1.0 %, and Melamphaidae, 0.9 %. About one-third of the remaining larvae were too poorly preserved (disintegrated) to identity.

On the basis of larval abundance, the dominant orders of fishes in oceanic waters are the Myctophiformes and Salmoniformes, making up between 85 and 88 %; the latter value assumes a proportionate representation of larvae of these groups in the "disintegrated" category, i.e., larvae too damaged or disintegrated to identify with certainty. Despite the dominance of fishes of the above two orders, a number of other groups of fishes are represented in the oceanic pelagic fish fauna. The berycoid fishes are rep-

TABLE 8.—Occurrences and counts of fish larvae taken in oblique 1.0-m plankton hauls on EASTROPAC I, summarized by family or larger grouping and by research vessel.

Family or larger grouping	Basic station data contained in Appendix Table no.		*Argo* 11.000 series		*David Starr Jordan* 12.000 series		*Rackaway* 13.000 series		*Alaminos* 14.000 series		Total EASTROPAC 1	
	By family or larger grouping	By genus or species	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae
[1]*1 Clupeidae	4		0	0	1	1	3	5	6	75	10	81
*2 Engraulidae	4		0	0	1	2	2	20	7	183	10	205
*3 Argentinidae		3	8	12	22	54	11	19	2	2	43	87
*4 Bathylagidae	1	3	49	284	73	496	93	1,075	89	3,025	304	4,880
*5 Gonostomatidae	1	3	118	4,663	118	4,782	135	8,129	88	4,472	459	22,046
*6 Sternoptychidae	1		90	923	74	1,712	106	2,161	67	891	337	5,687
*7 Astronesthidae	1		5	6	4	4	1	1	2	2	12	13
*8 Chauliodontidae	1		19	37	23	75	20	30	18	23	80	165
*9 Idiacanthidae	1		42	147	46	395	46	311	33	107	167	960
*10 Other Stomiatoidei	1	3	41	77	58	155	60	170	44	100	203	502
*11 Synodontidae	4		0	0	1	1	2	2	7	38	10	41
*12 Chlorophthalmidae			0	0	0	0	1	4	0	0	1	4
*13 Myctophidae	1	2	118	6,993	121	11,274	137	15,229	96	11,417	472	44,913
*14 Paralepididae	1	3	67	242	97	558	82	523	44	325	290	1,648
*15 Evermannellidae	3		14	20	4	6	9	12	0	0	27	38
*16 Scopelarchidae	1		35	64	46	104	39	120	22	41	142	329
*17 Scopelasauridae		3	2	2	3	3	1	1	3	10	9	16
18 Other Myctaphifarmes			7	8	2	2	1	2	2	2	12	14
19 Giganturidae			1	1	0	0	0	0	0	0	1	1
*20 Eel leptocephali	1		19	46	15	22	23	38	30	73	87	179
*21 Melamphaidae	1		66	165	77	171	96	321	59	200	298	857
22 Holacentridae			0	0	0	0	3	4	1	1	4	5
*23 Bregmacerotidae	1		62	291	56	825	56	435	21	254	195	1,805
24 Macrouridae			0	0	0	0	1	1	2	2	3	3
25 Scomberesocidae			0	0	0	0	1	1	0	0	1	1
*26 Exocoetidae	1	3	9	15	26	65	28	75	15	34	78	189
27 Trachypteridae	3		13	13	14	16	8	8	10	10	45	47
*28 Gempylidae-Trichiuridae	1	5	19	26	18	28	31	103	35	74	103	231
*29 Scombridae	1	3	26	237	44	342	69	605	46	735	185	1,919
*30 Istiophoridae			2	2	0	0	0	0	0	0	2	2
31 Ammodytidae			0	0	0	0	3	4	4	4	7	8
*32 Apogonidae		3	35	115	15	64	9	23	2	2	61	204
33 Balistidae			0	0	0	0	1	1	1	2	2	3
34 Bramidae	1		28	46	9	10	13	14	9	11	59	81
35 Callionymidae			0	0	0	0	0	0	8	12	8	12
*36 Carangidae	4	3	1	1	7	12	4	84	19	86	31	183
37 Carapidae			0	0	0	0	1	1	2	2	3	3
38 Champsodontidae			5	6	0	0	0	0	0	0	5	6
39 Chiasmodontidae	1		15	43	11	28	20	40	18	30	64	141
*40 Coryphaenidae		3	24	29	23	36	19	25	20	28	86	118
41 Gobiidae	4		0	0	7	17	23	262	30	251	60	530
42 Labridae	4		0	0	3	4	6	8	19	28	28	40
43 Mugilidae			0	0	0	0	0	0	5	9	5	9
*44 Nomeidae	1		39	178	52	346	51	291	36	146	178	961
45 Ophidiidae			1	1	1	1	0	0	4	18	6	20
46 Polynemidae			0	0	0	0	2	3	3	8	5	11
47 Sciaenidae			0	0	1	6	2	4	1	2	4	12
48 Scorpaenidae	4		1	1	5	8	15	41	27	113	48	163
49 Serranidae	4		0	0	3	15	3	53	20	184	26	252
50 Sphyraenidae			0	0	0	0	2	2	1	1	3	3
*51 Tetraganuridae		3	4	5	0	0	2	2	0	0	6	7
52 Triglidae			0	0	0	0	2	4	4	19	6	23
53 Uranoscapidae			0	0	0	0	0	0	1	1	1	1
*54 Bothidae		6	0	0	3	14	24	106	29	79	56	199
*55 Cynoglossidae		6	0	0	5	17	21	102	37	185	63	304
56 Laphiiformes	3		31	48	34	55	37	69	38	95	140	267
57 Other, including unidentified	1		69	257	56	323	54	436	57	523	236	1,539
58 Disintegrated larvae	1		89	515	97	837	90	400	73	1,389	348	3,141
Total			119	15,519	121	22,886	139	31,380	[2]99	25,324	[2]478	95,109

[1] Categories preceded by an asterisk are discussed in the text.
[2] No fish larvae were taken in four hauls of 14.000 series, hence total number of oblique 1.0-m collections was 482.

resented by Melamphaidae, a family of fishes that is almost as ubiquitous as the myctophids or gonostomatids. Fishes of the gadoid family, Bregmacerotidae, also are widely distributed in the warmer waters of all oceans. Among the ubiquitous epipelagics are the flyingfishes, Exocoetidae.

Only a moderate number of perciform fishes are widely distributed in offshore, oceanic waters. Among the more important are fishes of the families Scombridae, Gempylidae, Trichiuridae, Istiophoridae, Coryphaenidae, Bramidae, Nomeidae, Apogonidae, Chiasmodontidae, and Tetragonuridae.

Larvae of some demersal fishes have a much wider offshore distribution than one would associate with the known distribution of adults. Included in this group are larvae of bothid and cynoglossid flatfishes, and larvae of Scorpaenidae, Gobiidae, and Labridae.

Another widely distributed group in oceanic waters are the bizarre ceratioid fishes. The rotund larvae of these fishes were taken in about 30 % of the EASTROPAC collections, always in small numbers.

The basic data on the kinds and numbers of fish larvae obtained in the 482 EASTROPAC I collections are contained in six appendix tables, whose contents are summarized below, and keyed to Table 8 and to other tables in this report.

Appendix Table 1.—Counts of fish larvae, tabulated by family, for all stations occupied on EASTROPAC I. This table contains 22 categories, mostly families, but for completeness, a category is included for "other identified larvae," one for "unidentified larvae" and one for "disintegrated larvae" (i.e., larvae too damaged or disintegrated to identify with any certainty).

Appendix Table 2.—Myctophid larvae, tabulated by genus or species, for all stations occupied on EASTROPAC I. Myctophid larvae are tabulated by species for 12 kinds, and by genus for 8 kinds. Also included are categories for unidentified myctophids, and total myctophids. A summary of this appendix table is contained in Table 15.

Appendix Table 3.—Counts of selected categories of fish larvae by station. Table contains 23 categories including 10 species, 10 genera, 2 families, and 1 suborder; 9 of these were included in the category "other identified larvae" in Appendix Table 1.

Appendix Table 4.—Summary of occurrences and numbers of larvae of eight families limited in distribution to a broad coastal band or around offshore islands. Only positive stations are included. These eight families also were included in the category "other identified larvae" in Appendix Table 1.

Appendix Table 5.—Numbers and kinds of larvae of Gempylidae-Trichiuridae obtained in EASTROPAC I collections. Only positive stations are included. A summary of this appendix table is given in Table 19.

Appendix Table 6.—Numbers and kinds of flatfish (Pleuronectiformes) larvae obtained in EASTROPAC I collections. Only positive hauls are included. A summary of this appendix table is given in Table 22.

Appendix Table 7.—Standardized haul factors for the 482 oblique 1-m net hauls taken on EASTROPAC I. These factors adjust original counts of larvae to the comparable standard of numbers of larvae in 10 m^3 of water strained per meter of depth fished.

I will not attempt to comment on all 58 categories (family or larger grouping) summarized in Table 8, but will limit my discussion to 31 of these. In order to tie the text discussion closely to this table, I retain the numbers for categories as given in Table 8; those discussed in the text are preceded by an asterisk in this table.

COMMENTS ON LARVAE OF THE MAJOR FISH FAMILIES COLLECTED ON EASTROPAC I

1. CLUPEIDAE
(10 occurrences, 81 larvae)

Three species of clupeid larvae were taken in EASTROPAC I collections—*Opisthonema* sp.

(5 occurrences, 12 larvae), *Etrumeus acuminatus* Gilbert (2 occurrences, 6 larvae), and *Sardinops sagax* (Jenyns) (3 occurrences, 63 larvae). The latter two species were collected in the vicinity of the Galápagos Islands.

2. ENGRAULIDAE
(10 occurrences, 205 larvae)

The majority of the engraulids (5 occurrences, 174 specimens) were those of the Peruvian anchovy, *Engraulis ringens* Jenyns, collected at coastal stations between lat 6° and 13.5° S. Although larvae from only a few surface hauls have been sorted as yet, one haul was outstanding: the surface tow taken at station 14.069 contained 10,466 larvae and transforming specimens of Peruvian anchovy, *E. ringens*. Specimens ranged in size from 3.5 to 37.5 mm; most were between 4.0 and 7.5 mm in length but even transforming specimens, 20.0 to 37.5 mm long, were rather common (83 individuals). In the oblique 1-m haul at this station, 97 anchovy larvae were obtained.

3. ARGENTINIDAE
(43 occurrences, 87 larvae)

Three kinds of argentinid larvae were obtained: *Argentina* sp. (1 specimen), *Nansenia* sp. A (84 larvae), and *Nansenia* sp. B. (2 larvae). The specific identities of the two kinds of *Nansenia* larvae are still uncertain. On EASTROPAC I, *Nansenia* sp. A was taken most commonly in an equatorial band between lat 5° N and 5° S (Fig. 2). Larvae of *Nansenia* sp. A also occur in the southern portion of the area surveyed on cruises of CalCOFI, particularly to the south of Point San Eugenio, Baja California. A *Nansenia* larva with markedly different pigmentation pattern was obtained at station 11.154 in the central water mass of the South Pacific. A similarly pigmented *Nansenia* larva was obtained on NORPAC from the central water mass of the North Pacific.

4. BATHYLAGIDAE
(304 occurrences, 4,880 larvae)

Although two kinds of *Bathylagus* larvae were obtained, one species was taken in only two contiguous southern stations, 12.142 and 12.144. The eyes of the latter were carried on short stalks. The distribution of larvae of the commonly occurring species, *B. nigrigenys* Parr (296 occurrences, 2,987 larvae), was almost identical with that of the myctophid, *Diogenichthys laternatus* (Garman) (Fig. 3). The larvae of neither species occurred in the central South Pacific water mass; on the four outer lines, surveyed by *Argo* and *Jordan*, the occurrences of *B. nigrigenys* larvae ended at about lat 5° S. In the portion of the EASTROPAC area in which larvae of this species were distributed, they occurred in three-fourths of the stations occupied.

In the innermost pattern occupied by *Alaminos*, larvae of *Leuroglossus stilbius urotranus* (Bussing, 1965) were common (37 occurrences, 1,890 larvae). All but four specimens were obtained between lat 10° N and 10° S, and most within 300 miles of the coast (Fig. 2).

5. GONOSTOMATIDAE
(459 occurrences, 22,046 larvae)

Areal occurrence and relative abundance of gonostomatid larvae on EASTROPAC I are summarized in Table 9. They were obtained in 95 % of the hauls and made up approximately 23.2 % of the larvae.

As noted earlier, gonostomatid larvae were markedly more abundant in night hauls than in day hauls: 4.35 times as many, on the average. In contrast, larvae of the closely related hatchetfishes, Sternoptychidae, were taken in only slightly larger numbers at night (1.24 times as many as in day hauls). In the section dealing with depth distribution of fish larvae it was pointed out that the gonostomatid, *Vinciguerria* spp. occurred no deeper than ca. 130 m in NORPAC collections, whereas sternoptychid larvae were inhabitants of the aphotic zone below 130 m. An interesting exception should be noted: gonostomatid larvae of the subfamily Maurolicinae had depth distributions similar to sternoptychid larvae on NORPAC. Larvae of two Maurolicinae, *Maurolicus* and *Araiophos*, genera were taken on EASTROPAC. Although the depth distribution of these genera has not

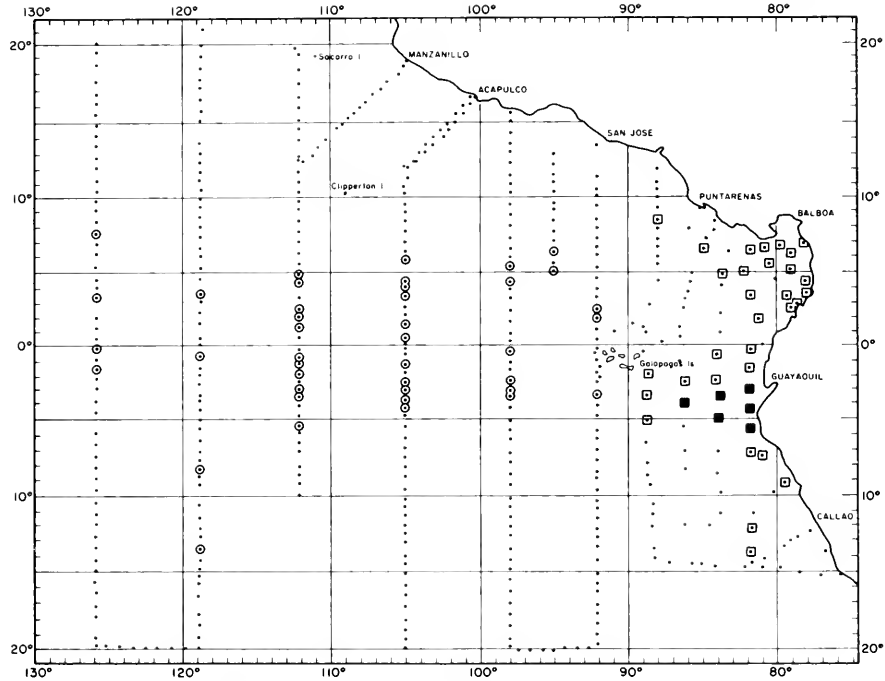

FIGURE 2.—Distribution of larvae of the argentinid, *Nansenia* spp., and of the bathylagid, *Leuroglossus stilbius urotranus* (Bussing) on EASTROPAC I. Records of occurrence of *Nansenia* larvae are shown as open circles with dot in center, while those of *Leuroglossus* larvae are open squares with dot (1 to 100 larvae) or closed squares (101 to 490 larvae). Small solid circles represent other stations occupied on EASTROPAC I.

TABLE 9.—Areal occurrence and relative abundance of larvae of Gonostomatidae on EASTROPAC I.

Latitude	*Argo* 11.000 series		*David Starr Jordan* 12.000 series		*Rockaway* 13.000 series		*Alaminos* 14.000 series		Total EASTROPAC I		
	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae	Average no. larvae per positive haul
20° N-15° N	16	418	20	1,534	5	115	--	--	41	2,067	50.4
15° N-10° N	14	380	22	745	24	607	--	--	60	1,732	28.9
10° N- 5° N	13	185	13	242	27	2,085	14	417	67	2,929	43.7
5° N- 0°	14	2,112	15	637	14	1,825	27	1,882	70	6,456	92.2
0° - 5° S	14	409	18	912	14	1,577	16	1,036	62	3,934	63.5
5° S-10° S	13	202	14	161	14	799	10	647	51	1,809	35.5
10° S-15° S	14	635	8	368	15	524	21	490	58	2,017	34.8
15° S-20° S	20	322	8	183	22	597	--	--	50	1,102	22.0
Total	118	4,663	118	4,782	135	8,129	88	4,472	459	22,046	48.0

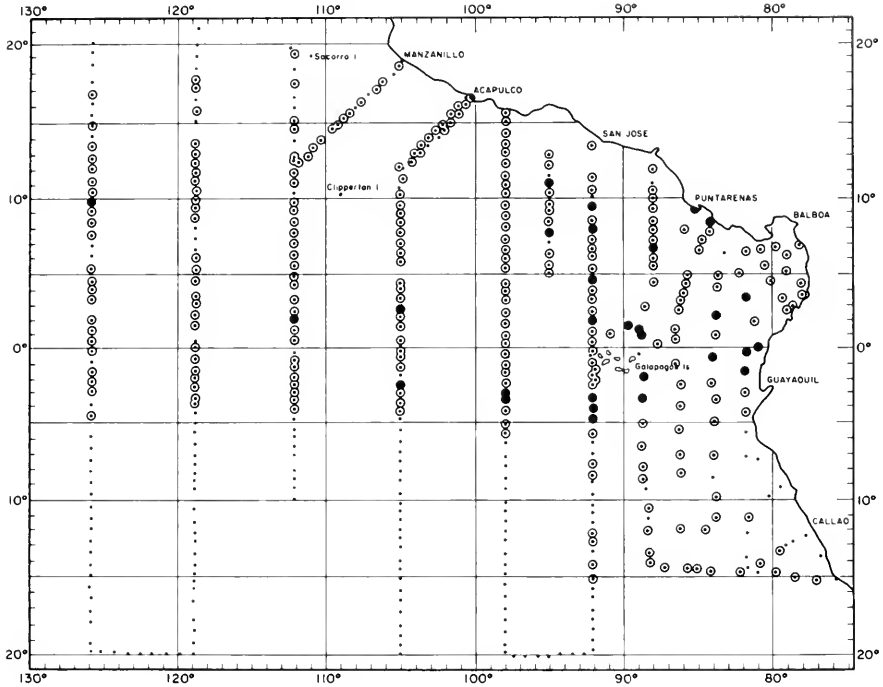

FIGURE 3.—Distribution of larvae of *Bathylagus nigrigenys* Parr on EASTROPAC I. Two orders of abundance are shown: open circles with dot in center represent counts of 1 to 25 larvae, large solid circles represent counts of 26 or more larvae. Small solid circles represent negative hauls.

been determined, they were sampled more fully during daylight hours than other gonostomatids; the night/day ratio for *Maurolicus* and *Araiophos* larvae was ca. 1.6 and 2.0 respectively.

Larvae belonging to six gonostomatid genera were common to abundant (Table 10) and larvae of several additional genera were taken occasionally. Larvae of two genera were of outstanding importance in the EASTROPAC area—*Vinciguerria* and *Cyclothone*. *Vinciguerria occurred* in 87.5 % of the collections, *Cyclothone* in 62.4 %.

Charts showing the distribution and relative abundance of larvae of Gonostomatidae and Sternoptychidae (combined) on EASTROPAC I will be included in the EASTROPAC Atlas.

Araiophos eastropas Ahlstrom and Moser (18 occurrences, 529 larvae)

Larvae of *Araiophos eastropas* were obtained only on the outermost pattern to the south of lat 10° S (Fig. 4). Within this limited area it was the most common gonostomatid. The species taken on EASTROPAC represented an undescribed species in a genus that previously

TABLE 10.—Frequency of occurrence and relative abundance of the kinds of gonostomatid larvae on EASTROPAC I.

Gonostomatid larvae	*Argo* 11.000 series		*David Starr Jordan* 12.000 series		*Rockaway* 13.000 series		*Alaminos* 14.000 series		Total EASTROPAC 1	
	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae
Araiophos eastropas	18	529	0	0	0	0	0	0	18	529
Cyclothone spp.	94	697	71	582	89	735	47	167	301	2,181
Diplophos taenia	18	51	40	107	14	24	1	1	73	183
Ichthyococcus spp.	7	9	11	16	18	31	5	5	41	61
Maurolicus muelleri	0	0	11	43	19	143	13	78	43	264
Vinciguerria spp.	96	3,339	109	4,011	131	7,179	86	4,211	422	18,740
Other gonostomatids	13	38	9	23	12	17	8	10	42	88
Total	118	4,663	118	4,782	135	8,129	88	4,472	459	22,046

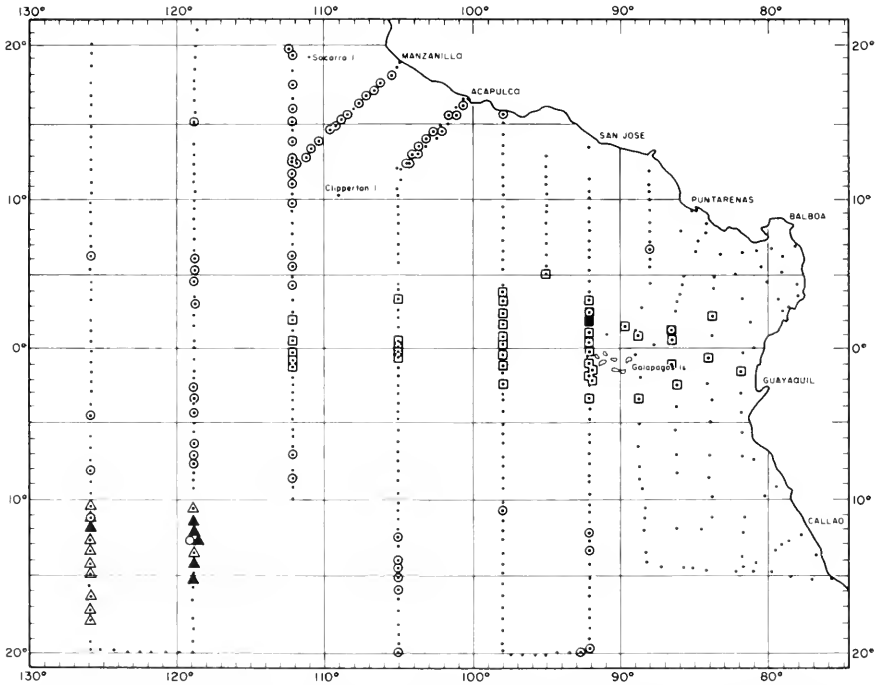

FIGURE 4.—Distribution of larvae of three species of Gonostomatidae on EASTROPAC I. Records of occurrence of larvae of *Araiophos eastropas* Ahlstrom and Moser are shown as triangles, *Diplophos taenia* (Günther) as large open circles, and *Maurolicus muelleri* (Gmelin) as squares. Solid triangles and squares are for counts of 26 or more larvae. Small solid circles represent negative hauls.

was known from a single collection made off Hawaii (Grey, 1961). Adults and larvae were described by Ahlstrom and Moser (1969).

Cyclothone spp. (301 occurrences, 2,181 larvae)

Larvae of *Cyclothone* spp. were taken least frequently in the northern quarter of the EASTROPAC pattern (betweeen lat 10° and 20° N, and in the inner pattern occupied by *Alaminos* (Table 11 and Fig. 5). In the former area, less than 20 % of the hauls (20 of 103) contained *Cyclothone* larvae; in the inshore pattern only about 45 % of the hauls (47 of 103) contained *Cyclothone* larvae. Over the remainder of the EASTROPAC I pattern *Cyclothone* larvae occurred at most stations (234 of 276). The lowest number of larvae per positive haul, 2.15 larvae, was obtained in the northern section; the next lowest, 3.55 larvae per positive haul, in the *Alaminos* pattern. Over the remainder of the pattern, 8.42 larvae were obtained per positive haul.

No attempt was made to identify the larvae of *Cyclothone* to species, and our hauls did not extend deep enough to collect adults.

Diplophos taenia Günther (73 occurrences, 183 larvae)

A study was made of larval and adult specimens of *Diplophos* in an attempt to determine whether the Pacific specimens should be assigned to *D. taenia* or retained as a distinct species, *D. pacificus* Günther. Grey (1960) had placed Pacific specimens in *D. taenia* but later she (Grey, 1964, p. 89) developed reservations because of the consistently lower photophore count of the ventral series in Pacific specimens. Without detailing my observations on *Diplophos*, which I plan to publish separately, I have concluded that our eastern Pacific *Diplophos* is not separable from the Atlantic *D. taenia*.

Larvae of *Diplophos* were taken most commonly to the north of lat 10° N—36 occurrences, 105 larvae (Fig. 4). The remaining 37 occurrences, 78 larvae were distributed throughout the EASTROPAC I pattern.

Ichthyococcus spp. (41 occurrences, 61 larvae)

Two kinds of *Ichthyococcus* larvae were taken on EASTROPAC I. The specific identity of the more common form has been determined as *I. irregularis* Rechnitzer and Böhlke; the other form has yet to be identified to species.

Maurolicus muelleri (Gmelin) (43 occurrences, 264 larvae)

Larvae of this species were taken only on an equatorial band between lat 5° N and 5° S and were not taken in the outer pattern occupied by *Argo* (Fig. 4). This distribution, without additional information, could be misleading. *Maurolicus* is known to have a wide latitudinal distribution in the South Pacific. For example, *Maurolicus* larvae were obtained at lat 33° S on MARCHILE VI, the portion of EASTROPAC II occupied by the Chilean vessel *Yelcho*. We also have collections from south of New Zealand, obtained on an *Eltanin* cruise. The species may be carried northward off South America in the Humboldt Current and then offshore in the equatorial current system.

TABLE 11.—Areal occurrence and relative abundance of larvae of *Cyclothone* spp. on EASTROPAC I.

Latitude	*Argo* 11.000 series		*David Starr Jordan* 12.000 series		*Rockaway* 13.000 series		*Alaminos* 14.000 series		Total EASTROPAC I		
	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae	Average no. larvae per positive haul
20° N-10° N	12	31	4	8	4	4	--	--	20	43	2.2
10° N- 0°	24	136	25	137	33	235	23	69	105	577	5.5
0° -10° S	24	179	29	246	20	117	13	43	86	585	6.8
10° S-20° S	34	351	13	191	32	379	11	55	90	976	10.8
Total	94	697	71	582	89	735	47	167	301	2,181	7.2

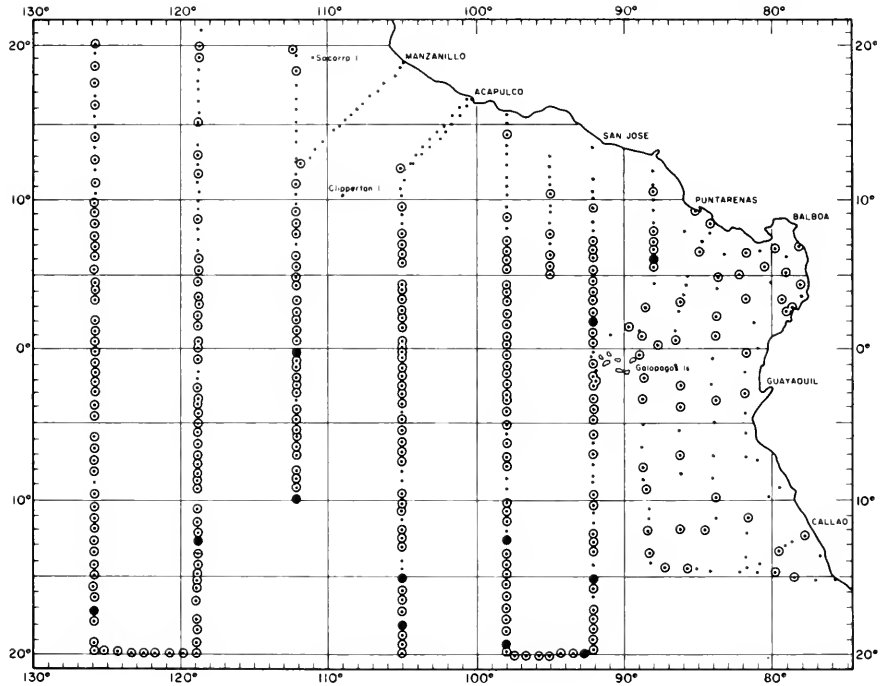

FIGURE 5.—Distribution of larvae of the gonostomatid *Cyclothone* spp. on EASTROPAC I. Collections of 1 to 25 larvae are shown as circles with dot in center, collections of 26 or more larvae as large solid circles; negative hauls are shown as small solid circles.

***Vinciguerria* spp. (422 occurrences, 18,740 larvae)**

Larvae of *Vinciguerria* occurred in more hauls than those of any other genus and ranked second in abundance to the myctophid genus *Diogenichthys*. The distribution of *Vinciguerria* larvae is shown in Figure 6. Although most of the material unquestionably is *V. lucetia* (Garman), some of the collections from offshore and particularly from the central South Pacific water mass between lat 5° and 20° S represent *V. nimbaria* (Jordan and Williams). The larvae of *V. nimbaria* are indistinguishable from those of *V. lucetia* (Ahlstrom and Counts, 1958), hence identification must be made on metamorphosing specimens, juveniles, and adults. The two species are closely allied, but readily separable from *V. poweriae* (Cocco) and *V. attenuata* (Cocco), the other two species of *Vinciguerria*, at all stages of development. A trenchant difference between the two "pairs" of species is the development of a pair of symphyseal photophores under the lower jaw in *V. lucetia* and *V. nimbaria* and the absence of this pair in *V. poweriae* and *V. attenuata*. The two characters most readily used for distinguishing

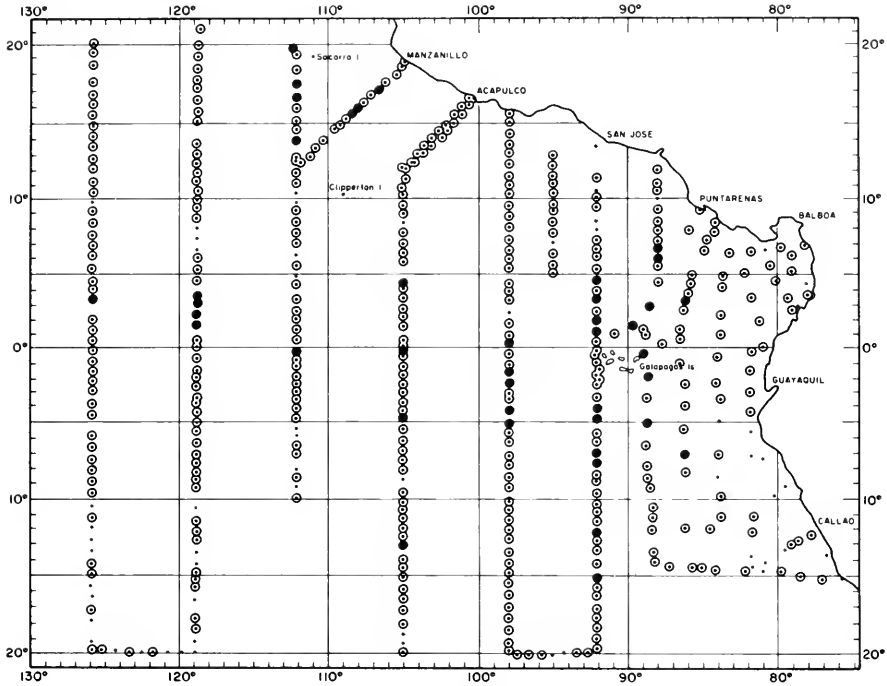

FIGURE 6.—Distribution of larvae of the gonostomatid, *Vinciguerria* spp. on EASTROPAC I. Collections of 1 to 100 larvae are shown as circles with dot in center, collections of 101 or more larvae as large solid circles; negative hauls are shown as small solid circles.

between *V. lucetia* and *V. nimbaria* are (1) number of gill rakers and (2) number of IV (and OV) photophores. Material of *V. nimbaria* studied from the eastern North Pacific (ibid.) had 5 to 6 + 15 gill rakers and 23 to 24 IV photophores (13 to 14 OV photophores) whereas *V. lucetia* had 8 to 10 + 18 to 23 gill rakers and 20 to 23 IV photophores (10 to 13 OV photophores). In the EASTROPAC area, *V. lucetia* maintained the high gill raker counts, but usually had 21 IV (11 OV) photophores. The offshore form referred to *V. nimbaria* usually had 22 IV (12 OV) photophores (1 less per group than in *V. nimbaria* from the temperate North Pacific) and 6 to 7 + 15 to 16 gill rakers (a slightly higher count).

In most areas the adults of the two species of *Vinciguerria* did not co-occur, hence the larvae can be assigned with some assurance to one or the other. For example, all collections made between lat 5° and 20° S from *Argo* and *Jordan* patterns were exclusively *V. nimbaria*. On these patterns the plankton hauls were supplemented by micronekton net hauls, and the latter contained material of *Vinciguerria* juveniles and adults from most stations occupied

at night. Unfortunately, the micronekton net was not used on *Rockaway* (12.000 series), and insufficient numbers of older stages (metamorphosing specimens and juveniles) were taken in plankton hauls to permit a meaningful separation of the two species in waters to the south of lat 5° S in this series.

Vinciguerria poweriae (Cocco) co-occurred with *V. nimbaria* in the central water mass of the North Pacific (Ahlstrom and Counts, 1958), but no material of *V. poweriae* was obtained in EASTROPAC collections. However, material of *V. attenuata* (Cocco) was obtained from farther south in the eastern Pacific on the "Downwind Expedition"—hence all four species of *Vinciguerria* do occur in the eastern Pacific.

Other gonostomatids (42 occurrences, 88 larvae)

Included in this category are larvae of two identified genera, *Gonostoma* and *Woodsia*, and several kinds of larvae that are unmistakably gonostomatid, but not identified as to kind.

6. STERNOPTYCHIDAE
(337 occurrences, 5,687 larvae)

Hatchetfish larvae ranked third in abundance (5.98 % of total), exceeded by larvae of Myctophidae and Gonostomatidae. The majority of hatchetfish larvae were those of *Sternoptyx diaphana* Hermann, and most of the remainder of *Argyropelecus lychnus* Garman. Because larvae of Sternoptychidae are more fragile than most other kinds and are usually in poor condition, no attempt was made to identify them to genus or species. Areal occurrence and relative abundance of sternoptychid larvae on EASTROPAC I are summarized in Table 12. Larvae were not only taken in markedly more collections between lat 10° N and 10° S—94 % of the collections were positive as compared with only 41 % in the remainder of the pattern—but more larvae were taken per positive haul—21.1 larvae as compared with 5.2.

7. ASTRONESTHIDAE
(12 occurrences, 13 larvae)

Several kinds of astronesthid larvae were collected in the EASTROPAC area: only one kind had heavy pigmentation on the body; the others were lightly, but characteristically pigmented. Larvae of Astronesthidae are similar in appearance to other stomiatoid larvae; they have a slender, elongated body, and a long intestine that underlies the body for about 7/10 or more of the standard length, and usually has a free terminal, trailing portion that can be quite long, often trailing beyond the caudal fin. Astronesthid larvae can be distinguished readily from other stomiatoid larvae by the forward position of the dorsal fin in relation to the anal fin. Developmental series of astronesthid larvae have not been described in literature. Eleven of the 12 occurrences of astronesthid larvae were taken within 10° ± of the equator.

8. CHAULIODONTIDAE
(80 occurrences, 165 larvae)

Larvae of *Chauliodus* are readily identifiable to genus, but are difficult to separate at the spe-

TABLE 12.—Areal occurrence and relative abundance of larvae of Sternoptychidae on EASTROPAC I.

Latitude	*Argo* 11.000 series		*David Starr Jordan* 12.000 series		*Rockaway* 13.000 series		*Alaminos* 14.000 series		Total EASTROPAC I		
	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae	Average no. larvae per positive haul
20° N-15° N	8	44	0	0	0	0	--	--	8	44	5.5
15° N-10° N	6	41	3	31	9	66	--	--	18	138	7.7
10° N- 5° N	14	312	14	479	29	1,006	14	237	71	2,034	28.6
5° N- 0°	14	133	15	430	13	456	22	414	64	1,433	22.4
0° - 5° S	14	140	18	353	14	303	16	129	62	925	14.9
5° S-10° S	12	198	14	317	14	210	10	104	50	829	16.6
10° S-15° S	13	40	8	98	11	83	5	7	37	228	6.2
15° S-20° S	9	15	2	4	16	37	--	--	27	56	2.1
Total	90	923	74	1,712	106	2,161	67	891	337	5,687	16.9

cies level, because of lack of pigmentation. It has not been determined yet whether one or more species of *Chauliodus* occur in the EASTROPAC area. *Chauliodus* larvae were widely distributed, usually occurring singly (50 such occurrences). In only five hauls were six or more larvae obtained per haul; all of these were in the outer patterns occupied by *Jordan* and *Argo*.

9. IDIACANTHIDAE
(167 occurrences, 960 larvae)

It is not known definitely whether one or two species of *Idiacanthus* occur in the eastern Pacific; the problem hinges on whether *I. panamensis* is distinct from *I. antrostomus* Gilbert. Gibbs (1964) considered the two species to be "probably synonymous." In the EASTROPAC area, *Idiacanthus* occurred more frequently in the northern portion of the pattern, between lat 10° and 20° N, as is shown in Table 13.

10. OTHER STOMIATOIDEI
(203 occurrences, 502 larvae)

Larvae belonging to three families are included as other Stomiatoidei—i.e., of Stomiatidae, Melanostomiatidae, and Malacosteidae. The most common larva in this category, that of *Bathophilus filifer* (Garman) (86 occurrences, 227 larvae) is separately tabulated in Appendix Table 3. Larvae of *Eustomias*, representing several species, occurred in 17 collections. Larvae of *Stomias* were separately tabulated from only eight collections; however, a number of larvae tabulated as unidentified stomiatoid larvae undoubtedly are those of *Stomias*. According to Gibbs (1969), no fewer than three species of *Stomias* occur in the eastern Pacific. Many stomiatoid larvae were poorly preserved, and were not identifiable with any certainty.

11. SYNODONTIDAE
(10 occurrences, 41 larvae)

All but three specimens were taken in the inner pattern, occupied by *Alaminos*. Six of the seven occurrences in this pattern were at contiguous stations occupied off Ecuador and the Gulf of Panama (Fig. 7). Synodontidae are coastal forms. No attempt was made to identify the larvae to the species level.

12. CHLOROPHTHALMIDAE
(1 occurrence, 4 larvae)

The only record of *Chlorophthalmus* larvae was from station 13.052. Larvae in this sample ranged from 5.0 to 6.5 mm long. Pigmentation was limited to a large, single peritoneal pigment patch—and to a few small melanophores on the dorsal and ventral margin of the tail soon before the tip of the notochord. Two larger specimens of *Chlorophthalmus* were taken in the micronekton net hauls, a 23.0-mm specimen at station 14.018 and a 39.5-mm specimen at station 14.051. Pigment on both was limited to the peritoneal patch, and a midline melanophore over the hypural complex; otherwise both specimens were milky white, without scales. The larger specimen had the following fin counts: D. 11, A. 11, V. 9, P. 17. These are identical to counts given by Garman (1899) for his species, *C. mento* from the Gulf of Panama, to which our material probably is referable.

TABLE 13.—Areal occurrence and relative abundance of larvae of Idiacanthidae on EASTROPAC I.

Latitude	*Argo* 11.000 series		*David Starr Jordan* 12.000 series		*Rockaway* 13.000 series		*Alaminos* 14.000 series		Total EASTROPAC I		
	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae	Average no. larvae per positive haul
20° N-10° N	18	107	34	379	17	149	--	--	69	635	9.2
10° N- 0°	11	19	7	10	14	65	20	56	52	150	2.9
0° -10° S	4	6	2	4	7	44	4	9	17	61	3.6
10° S-20° S	9	15	3	4	8	53	9	42	29	114	3.9
Total	42	147	46	395	46	311	33	107	167	960	5.7

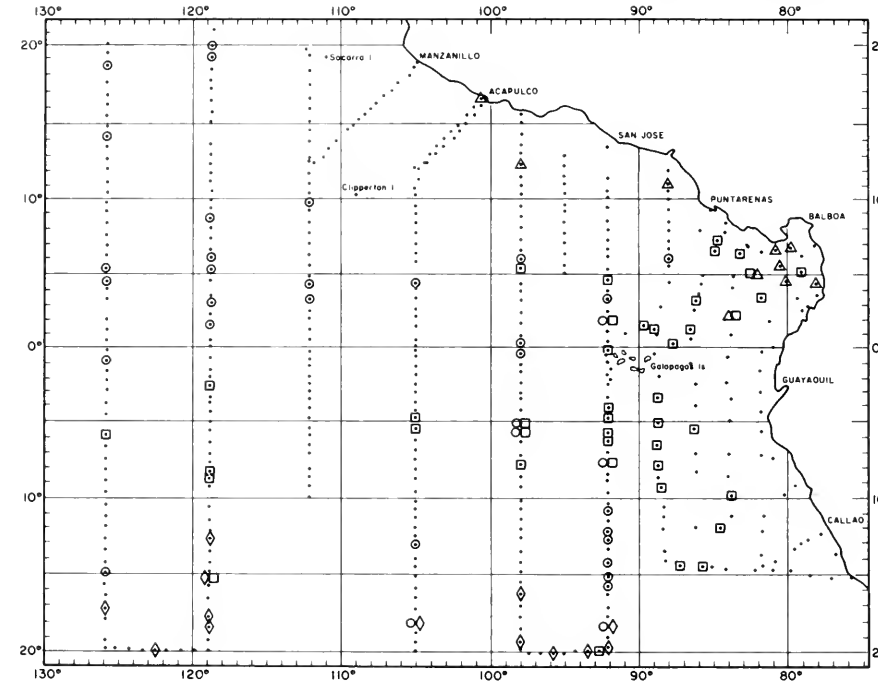

FIGURE 7.—Distribution of larvae of the paralepidids, *Macroparalepis macrurus* Ege and *Sudis atrox* Rofen, of *Synodus* spp., and of the gempylid *Nealotus tripes* Johnson on EASTROPAC I. Records of occurrence of larvae of *Macroparalepis macrurus* are shown as an open circle, larvae of *Sudis atrox* as a diamond, larvae of *Synodus* spp. as a triangle, and larvae of *Nealotus tripes* as a square; negative hauls are shown as small solid circles.

13. MYCTOPHIDAE
(472 occurrences, 44,913 larvae)

Myctophids made up 47.2% of the fish larvae taken on EASTROPAC I. Of the 482 oblique hauls taken on EASTROPAC I, 472 contained myctophid larvae. This dominant group occurred almost everywhere. However, as is shown in Table 14, larger numbers of myctophid larvae were taken per haul between lat 10° N and 5° S.

The myctophid fauna is a large one in numbers of genera and species represented in the eastern tropical Pacific. This diversity is shown in Table 15, in which occurrence and abundance of myctophid larvae are summarized by genus or species; the number of genera listed is 19. Even so, larvae of *Diogenichthys laternatus* made up over half of the total.

The study of larval myctophids is aided by the diversity of larval morphology found in this family, and by the fact that the larvae of most genera have a characteristic form that permits

identification to genus, even for genera in which the species composition has not been fully worked out. This point was stressed in two recent papers dealing with identification of myctophid larvae (Pertseva-Ostroumova, 1964; Moser and Ahlstrom, 1970).

Because of the importance of this group in the tropical ichthyoplankton, I will discuss its composition in more detail than for any other family except the Gonostomatidae.

Moser and Ahlstrom (1970) described developmental series for 14 species of lanternfishes with narrow-eyed larvae in the California Current. The following species also occur in the EASTROPAC area: *Electrona rissoi, Diogenichthys atlanticus, D. laternatus, Benthosema panamense, Hygophum atratum, H. reinhardti, Myctophum nitidulum, Loweina rara, Gonichthys tenuiculus,* and *Centrobranchus choerocephalus.*

TABLE 14.—Areal occurrence and relative abundance of larvae of Myctophidae on EASTROPAC I.

Latitude	*Argo* 11.000 series		*David Starr Jordan* 12.000 series		*Rockaway* 13.000 series		*Alaminos* 14.000 series		Total EASTROPAC I		
	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae	Average no. larvae per positive haul
20° N-15° N	16	430	20	1,000	5	116	--	--	41	1,546	37.7
15° N-10° N	14	568	23	1,444	24	2,826	--	--	61	4,838	79.3
10° N- 5° N	14	1,323	14	2,136	29	5,856	15	2,730	72	12,045	167.3
5° N- 0°	14	1,988	15	2,327	14	1,325	27	6,075	70	11,715	167.4
0° - 5° S	14	1,233	18	3,413	14	1,635	16	1,209	62	7,490	120.8
5° S-10° S	13	567	15	408	14	994	13	635	55	2,604	47.3
10° S-15° S	14	563	8	296	15	1,362	25	768	62	2,989	48.2
15° S-20° S	19	321	8	250	22	1,115	--	--	49	1,686	34.4
Total	118	6,993	121	11,274	137	15,229	96	11,417	472	44,913	95.2

TABLE 15.—Summary, by genus or species, of occurrences and relative abundance of myctophid larvae in the four vessel patterns occupied on EASTROPAC I.[1]

Myctophid larvae	*Argo* 11.000 series		*David Starr Jordan* 12.000 series		*Rockaway* 13.000 series		*Alaminos* 14.000 series		Total EASTROPAC I	
	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae
Benthosema panamense	0	0	1	63	5	918	1	46	7	1,027
Centrobranchus spp.	0	0	3	4	0	0	0	0	3	[2]4
Ceratoscopelus townsendi-complex	46	235	24	140	42	633	5	12	117	1,020
Diaphus spp.	62	490	96	1,363	72	949	21	71	251	2,873
Diogenichthys laternatus	69	2,202	89	5,259	92	9,089	89	8,775	339	25,325
Diogenichthys atlanticus	3	4	6	11	18	75	2	2	29	92
Electrona sp.	5	6	9	34	19	34	0	0	33	74
Gonichthys tenuiculus	5	8	20	56	39	101	28	67	92	232
Gonichthys sp.	0	0	0	0	0	3	0	0	3	3
Hygophum atratum & *H. reinhardti*	30	177	52	352	37	268	8	90	127	887
Hygophum proximum	67	611	30	215	19	72	0	0	116	898
Lampadena spp.	13	27	10	21	15	71	0	0	38	119
Lampanyctus spp.	99	1,240	96	2,063	107	1,347	74	1,232	376	5,882
Lepidophanes pyrsobolus-complex	7	26	14	109	10	22	3	6	34	163
Lobianchia sp.	2	14	2	3	12	22	0	0	16	39
Loweina rara	18	25	7	11	13	14	4	5	43	56
Myctophum spp.	52	624	48	323	47	160	40	286	187	1,393
Notolynchus valdiviae	40	210	47	290	60	344	11	24	158	868
Notoscopelus resplendens	13	37	21	104	21	73	15	69	70	283
Protomyctophum sp.	3	4	19	37	14	37	0	0	36	78
Symbolophorus evermanni	71	535	47	248	58	381	36	318	212	1,482
Triphoturus spp.	17	33	25	82	54	256	44	135	140	506
Unidentified myctaphid larvae	39	98	36	65	62	190	26	56	163	409
Disintegrated myctaphid larvae	75	387	60	423	64	170	42	223	241	1,203
Total myctaphid larvae	118	6,993	121	11,274	137	15,229	96	11,417	472	44,913

[1] The table summarizes the data presented by individual station in Appendix Table 2.
[2] *Centrobranchus* larvae are included under unidentified myctaphid larvae in Appendix Table 2.

Benthosema panamense (Tåning) (7 occurrences, 1,027 larvae)

The relatively large number of larvae taken in a few hauls probably results from the adults of this species occurring in more compact aggregations than other myctophids (Alverson, 1961). All occurrences were within a few hundred miles of the coast, mostly off Mexico and Costa Rica. Distribution of larvae of *B. panamense* in the eastern tropical Pacific was illustrated in Moser and Ahlstrom (1970).

Centrobranchus spp. (3 occurrences, 4 larvae)

The larvae assigned to *Centrobranchus* represent two kinds; one of these is identical to the larvae described as *C. choerocephalus* (Moser and Ahlstrom, 1970). The other is possibly *C. andrae*.

***Ceratoscopelus townsendi*-complex (117 occurrences, 1,020 larvae)**

Until recently, only two species of *Ceratoscopelus* were recognized: *C. townsendi* (Eigenmann and Eigenmann) and *C. maderensis* (Lowe). The larvae of these two species are distinctively different, especially in pigmentation. Nafpaktitis and Nafpaktitis (1969) concluded that *C. warmingi* (Lutken) was distinct from *C. townsendi* and was the more widely distributed species. They indicated that *C. townsendi* probably was restricted in its distribution to the eastern North Pacific. The major difference between the two species is the presence on *C. townsendi* of a large patch of luminous tissue along the dorsal rim of the orbit on specimens larger than ca. 21 mm SL; otherwise, the two species are almost identical in meristic characters, arrangement of photophores, and the placement of most luminous patches.

Subsequent to the publication of the paper by Nafpaktitis and Nafpaktitis (1969), my colleague, H. G. Moser, and I studied developmental series of *Ceratoscopelus* larvae previously assigned to *C. townsendi*. Moser (unpublished) studied eastern North Pacific material (CalCOFI and NORPAC) and material from the eastern South Pacific obtained on EASTROPAC I; I had the opportunity to examine a number of collections of *Ceratoscopelus* larvae collected by the *Meteor* in the Indian Ocean (through the generosity of W. Nellen of the Institut für Meereskunde, University of Kiel, Germany). Based on criteria of Nafpaktitis and Nafpaktitis, adults from both the Indian Ocean and southern portion of the EASTROPAC area were referable to *C. warmingi*, those from CalCOFI and NORPAC to *C. townsendi*. Larvae from the three regions were strikingly similar in appearance. Observed differences were mostly in rate of development, particularly in the sizes at which fin formation took place and at which photophores developed. Even so, somewhat greater differences were observed between *Ceratoscopelus* larvae from the Indian Ocean and those from the EASTROPAC area, than between larvae from the two eastern Pacific regions. For the present, I choose to call attention to the complexity of this problem by referring EASTROPAC material to the *C. townsendi*-complex.

Distribution of *C. townsendi*-complex larvae on EASTROPAC I is illustrated in Figure 8. Most occurrences were in offshore waters between lat 5° and 20° S, i.e., in the South Pacific central water mass. *Ceratoscopelus* larvae are known to have a complementary distribution in the eastern North Pacific. On the NORPAC Expedition *Ceratoscopelus* larvae were the dominant myctophid in the North Pacific central water mass between ca. lat 20° and 40° N. The occurrences of *Ceratoscopelus* larvae in the *Argo* pattern between lat 17° and 20° N are a fragment of this northern population. The few occurrences of *Ceratoscopelus* larvae in waters of the equatorial current system were small individuals. A few adults also were collected in this region, hence tropical waters may not be a barrier to the interchange of fish between the populations in the North and South Pacific.

Diaphus spp. (251 occurrences, 2,873 larvae)

Diaphus, the genus of myctophids with the largest number of species, is represented in the tropical eastern Pacific by a number of larval forms whose specific identities have been worked out only partially.

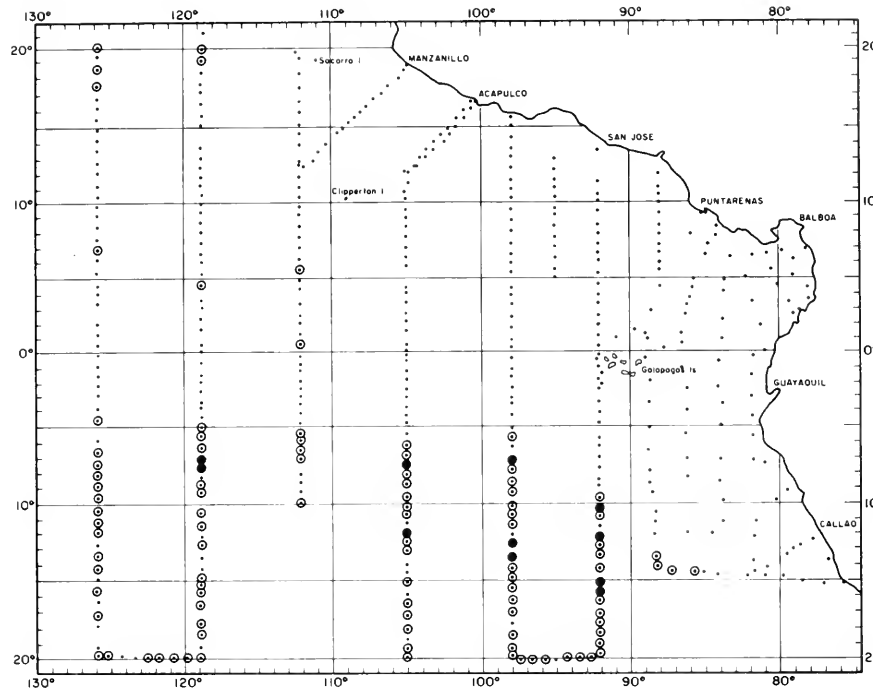

FIGURE 8.—Distribution of larvae of the myctophid, *Ceratoscopelus townsendi*-complex on EASTROPAC I. Collections of 1 to 25 larvae are shown as circles with dot in center, collections of 26 or more larvae as large solid circles; negative hauls are shown as small solid circles.

The genus *Diaphus* is not a natural assemblage, inasmuch as there are two distinctive larval morphs for the species in the EASTROPAC area. One group has slender-bodied larvae with persistent ventral midline pigment on the tail; the adults of this group possess both Vn and So ocular photophores (subgenus *Diaphus* of Fraser-Brunner, 1949). The other and larger group has stubby-bodied larvae which usually are but lightly pigmented; in the EASTROPAC area the larvae of *Diaphus pacificus* Parr is a representative example.

Although *Diaphus* larvae were distributed over most of the area covered on EASTROPAC I, they were least common in the inner pattern occupied by *Alaminos* (21 occurrences, 71 larvae) and most consistently taken in the intermediate pattern occupied by *Jordan* (96 occurrences, 1,363 larvae).

Diogenichthys laternatus (Garman) (339 occurrences, 25,325 larvae)

Although this is by far the most abundant kind of larva taken on EASTROPAC I, it did not occur in the central water mass of the South

Pacific (Fig. 9). This species similarly is absent from the central water mass of the North Pacific (Moser and Ahlstrom, 1970). There is a striking similarity in the distributions of larvae of *D. laternatus* and those of *Bathylagus nigrigenys* Parr (Fig. 3) in the EASTROPAC area. *D. laternatus* is one of the smaller species of myctophids, measuring only 20.0 to 30.0 mm as adults; hence its biomass probably is not as great as its larval abundance would suggest.

Diogenichthys atlanticus (Tåning)
(29 occurrences, 92 larvae)

In contrast to its cogener, larvae of *Diogenichthys atlanticus* were taken mostly in the central water mass of the South Pacific on EASTROPAC I (Fig. 9). Most of the occurrences were to the south of lat 10° S on three adjacent lines (along long 92°, 98°, and 115° W). Two occurrences at the southern end of the *Alaminos* pattern, however, indicate that this

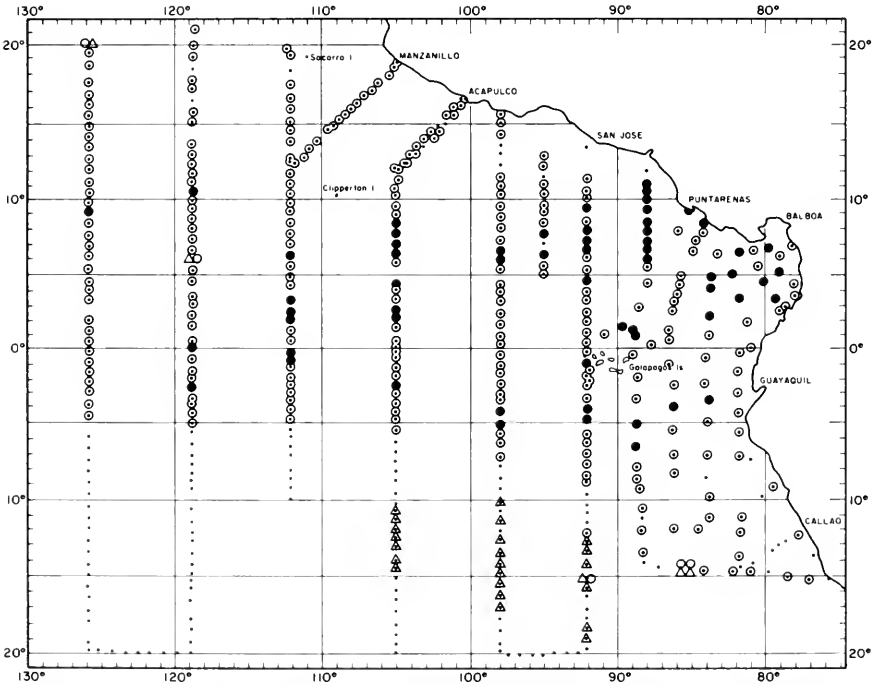

FIGURE 9.—Distribution of larvae of two species of myctophids of the genus *Diogenichthys* on EASTROPAC I. Records of occurrence of larvae of *D. atlanticus* (Tåning) are shown as triangles, records of occurrences of larvae of *D. laternatus* (Garman) as large circles with dot in center for hauls containing 0 to 100 larvae, and as large solid circles for hauls containing 101 or more specimens of this species; negative hauls are shown as small solid circles.

species is not restricted to the central water mass but also can occur in the transitional waters of the Humboldt Current. Larvae of this species were taken close to the Chilean coast between lat 20° and 30° S on MARCHILE VI, the Chilean contribution to EASTROPAC II. *D. atlanticus* appears to be a temperate-subtropical species, whereas *D. laternatus* is a tropical-subtropical species. The distribution of larvae of this species in the eastern North Pacific is given in Moser and Ahlstrom (1970, figs. 41 and 42). A larval specimen taken at lat 6° N along long 119° W shows that this species can bridge the tropical gap between its areas of usual occurrence in more temperate waters of the North and South Pacific.

Electrona sp. (33 occurrences, 74 larvae)

Distribution of *Electrona* larvae on EASTROPAC I was limited to two bands—one centering on lat 5° N (6 occurrences, 16 larvae) the other in the central water mass of the South Pacific, between lat 8° and 20° S (27 occurrences, 58 larvae). The *Electrona* larvae all resemble *E. rissoi*, although two kinds may be present.

Gonichthys tenuiculus (Garman) (92 occurrences, 232 larvae)

Larvae of *Gonichthys tenuiculus* have a similar distribution in the eastern tropical Pacific to those of *Diogenichthys laternatus*. Larvae of a different species of *Gonichthys* (3 occurrences, 3 larvae) were obtained at the southern end of the *Rockaway* pattern. Beebe and Vander Pyl (1944) reported collecting more adults of *G. tenuiculus* (reported as *Myctophum coccoi* (Cocco)), than of any other myctophid on the Arcturus Expedition to the eastern Pacific in 1925. Their collections were made on adults aggregating at the surface. Based on larval evidence, *Gonichthys tenuiculus* is only moderately common.

Hygophum atratum-reinhardti (127 occurrences, 887 larvae)

Larvae of these two species are similar in appearance and difficult to distinguish at some stages of larval development. Larvae of *Hygophum atratum* (Garman) were distributed over much of the EASTROPAC pattern; however some occurrences at the southern end of the patterns of *Rockaway*, *Jordan*, and *Argo* were referable to *H. reinhardti* (Lutken).

Hygophum proximum Becker (116 occurrences, 898 larvae)

Hygophum proximum is a truly oceanic species, not occurring at all in the inner pattern worked by *Alaminos*, and it was most abundant in the outer pattern occupied by *Argo* (Fig. 10). It occurs in the central water masses of the North and South Pacific, but also in the equatorial current system; the largest collection of larvae (103 specimens) was obtained at the equator.

Lampadena spp. (38 occurrences, 119 specimens)

Two and possibly three kinds of *Lampadena* larvae were obtained on EASTROPAC. A developmental series definitely has been established for only one species, *Lampadena urophaos* Paxton. The relatively few occurrences of *Lampadena* larvae on EASTROPAC I were mostly in the southern portion of the three outer vessels (24 of 38 occurrences) and most of the remainder in an offshore band lying between lat 4° and 8° N (9 occurrences).

Lampanyctus spp. (376 occurrences, 5,882 larvae)

Larvae of *Lampanyctus* were taken in more collections than those of any other myctophid genus but were not identified to species. A number of species of *Lampanyctus* occur in the EASTROPAC area, of which *L. idostigma* Parr, L. *omostigma* Gilbert, *L. parvicauda* (Parr), and *L. steinbecki* Bolin are among the more common. Larval series are being worked out for these.

Lepidophanes sp. (34 occurrences, 163 larvae)

The species of *Lepidophanes* that occur in the EASTROPAC area belong to the *Lepidophanes*

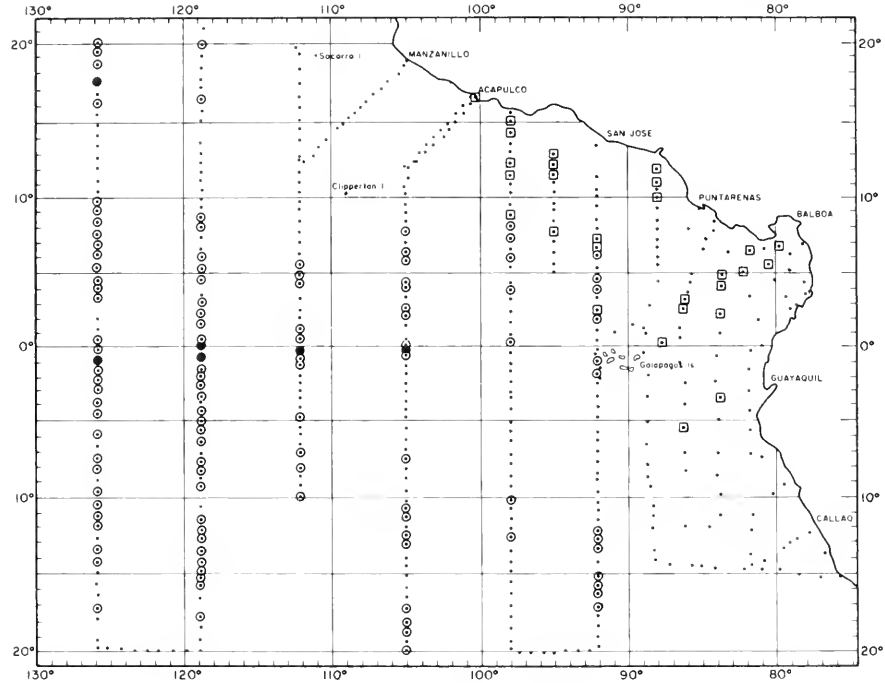

FIGURE 10.—Distribution of larvae of the myctophid *Hygophum proximum* (Becker) and of the bothid flatfish *Bothus leopardinus* (Günther) on EASTROPAC I. Records of occurrence of larvae of *H. proximum* are shown as open circles with dot in center for hauls containing 1 to 25 larvae, and as large solid circles for hauls containing 26 or more larvae; records of occurrence of larvae of *B. leopardinus* are shown as squares; negative hauls are shown as small solid circles.

pyrsobolus (Alcock) complex. Larvae of *Lepidophanes* are almost unpigmented, big eyed, and moderately deep bodied. They have few distinctive characters and can be confused with larvae of *Diaphus* and *Ceratoscopelus*. The majority of the records for *Lepidophanes* were of large larvae.

Lobianchia sp. (16 occurrences, 39 larvae)

Larvae of *Lobianchia* were not recognized until the identification of EASTROPAC larvae was well underway, hence our records of occurrences may be incomplete (some but not all samples were rechecked subsequently). The head of *Lobianchia* larvae is more massive than in most myctophid larvae. The most diagnostic feature, however, is the unusual manner in which the pectoral fins develop: the upper fin rays in each pectoral develop sooner than the remainder of the fin rays and become conspicuously elongated (Tåning, 1918). Twelve of the 16 occurrences were in the pattern worked by *Alaminos* and half of these were at adjacent

stations located between lat 6° and 2° N along long 92° W.

Loweina rara (Lutken) (43 occurrences, 56 larvae)

The larger larvae of *Loweina rara* are among the most elegant of myctophid larvae. Larvae of this species were rather uncommon in the EASTROPAC area, although widely distributed. Larvae were taken most frequently, however, in the vicinity of the equator, between ca. lat 8° N and 7° S; 36 of the 43 occurrences were in the equatorial zone. The largest collection of *Loweina* larvae was only four specimens, and only a single specimen was obtained in most collections (i.e., in 35 of 43). The distribution of larvae of *L. rara* on EASTROPAC I is illustrated in Moser and Ahlstrom (1970).

Myctophum spp. (187 occurrences, 1,393 larvae)

Myctophum is one of the more abundant genera represented in the eastern tropical Pacific. Juvenile and adults of five species were obtained in 1-m plankton hauls and micronekton net hauls: *Myctophum aurolaternatum* Garman, *M. asperum* Richardson, *M. brachygnathos* (Bleeker), *M. lychnobium* Bolin, and *M. nitidulum* Garman. Body form and pigmentation of the five of six kinds of *Myctophum* larvae taken in EASTROPAC I are as diverse as has been observed within a myctophid genus. Larvae of *M. nitidulum*, described by Moser and Ahlstrom (1970), are broad headed and deep bodied with eyes on short stalks; larger larvae of this species are among the most heavily pigmented myctophid larvae.

A quite different developmental pattern is displayed by larvae of *M. asperum* and *M. brachygnathos*. The larvae of these species are also deep bodied and big headed, but the eyes are not borne on stalks. The most characteristic feature of the development of these larvae is the early appearance of Dn photophores which form on larvae between 4.0 to 5.0 mm in length, soon after the appearance of the Br_2 photophores. Larvae of *M. asperum* develop large characteristic melanophores (Pertseva-Ostroumova, 1964), but larvae of *M. brachygnathos* are only slightly pigmented.

Larvae of *M. lychnobium* also are but lightly pigmented; they are much more slender and elongated than larvae of *M. brachygnathos* and do not develop the Dn photophores early. A notable feature is the marked length of the teardrop (choroid) tissue that develops under the eyes (as long as in *Gonichthys* or *Centrobranchus* larvae).

The extraordinary larvae of *M. aurolaternatum* were only recently recognized and are not included in the above counts of *Myctophum*.

Notolychnus valdiviae (Brauer) (158 occurrences, 868 larvae)

This is probably the smallest species of myctophid, and certainly one of the most widespread in offshore, oceanic waters. The larvae seldom occur in large numbers (average number per positive haul was 5.5 larvae). They were present in about one-third of the collections made on EASTROPAC I, although most occurrences were farther offshore than 300 miles of the coast (Fig. 11). Juvenile and adult *N. valdiviae* were frequently taken in the oblique plankton hauls. Perhaps as many juvenile and adult specimens of *N. valdiviae* were obtained by this means as of all other myctophids combined. Since this species has only a middling rank with regard to abundance of larvae, the frequency of capture of adults is probably less a measure of abundance than of their shallow depth distribution and poor swimming ability.

Notoscopelus resplendens (Richardson) (70 occurrences, 283 larvae)

This is the species of *Notoscopelus* known to occur in the eastern Pacific. On EASTROPAC, *Notoscopelus* larvae were taken more frequently and in larger numbers in the equatorial zone between lat 5° N and 5° S (40 occurrences, 209 larvae).

Protomyctophum sp. (36 occurrences, 78 larvae)

All occurrences of *Protomyctophum* larvae, except one, were between lat 10° N and 5° S.

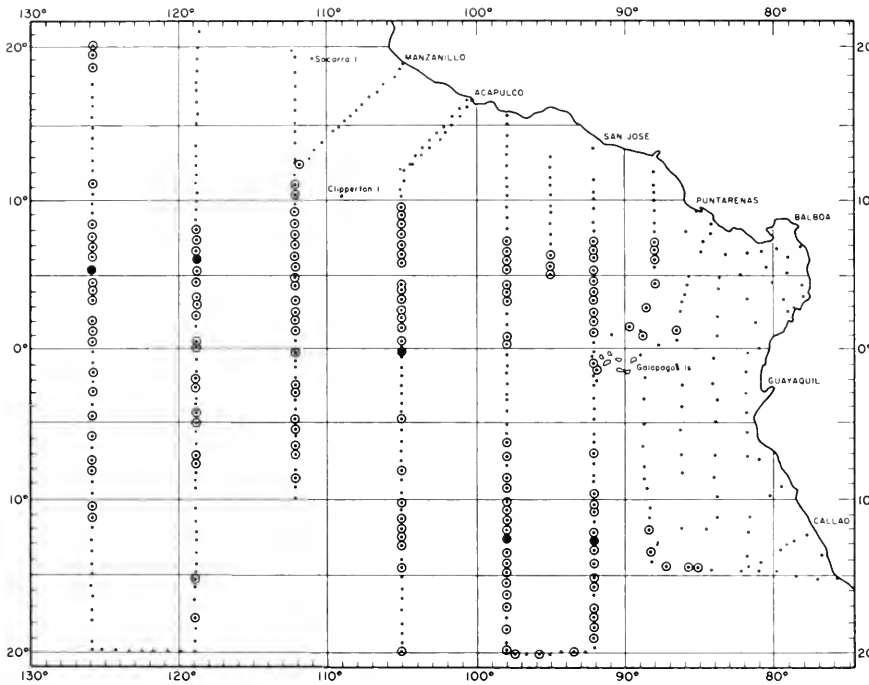

FIGURE 11.—Distribution of larvae of the myctophid, *Notolychnus valdiviae* (Brauer) on EASTROPAC I. Collections of 1 to 25 larvae are shown as circles with dot in center, collections of 26 or more larvae as large solid circles; negative hauls are shown as small solid circles.

Only one kind of *Protomyctophum* larva, belonging to the subgenus *Hierops*, was taken on EASTROPAC. The specific identity is unknown, as no juveniles or adults were obtained. The larva has a single lateral pigment spot per side over the gut, resembling in this respect the larva of *P. crockeri* (Bolin) (Moser and Ahlstrom, 1970). However, internal pigment develops over the hypural bones of the caudal complex in older larvae—resembling in this respect the pigmentation of older larvae of *P. thompsoni* (Chapman). The tropical form lacks ventral pigment on the tail posterior to the anus, such as is developed on larvae of *P. thompsoni*, and probably represents an undescribed species.

Symbolophorus evermanni (Gilbert) (212 occurrences, 1,482 larvae)

Only one kind of *Symbolophorus* larvae appears to be present in the EASTROPAC survey area, despite its distribution in different water masses including the central water mass of the South Pacific. Fewest occurrences were in the northern portion of the EASTROPAC pattern, between lat 10° and 20° N. The number of lar-

vae per positive haul ranged from 1 to 72 (average 7.0); 15 collections contained 25 or more larvae, most distributed between lat 7° and 20° S. Distribution of the larvae of *S. evermanni* on EASTROPAC I is illustrated in Figure 12.

Triphoturus spp. (140 occurrences, 506 larvae)

Larvae of at least two species of *Triphoturus* were taken in the EASTROPAC area. Of particular interest are larvae of *Triphoturus oculeus* (Garman); this species previously was considered a synonym of *T. mexicanus* (Gilbert), but larvae of the two species are differently pigmented. *T. oculeus* occurs in a broad coastal band between Panama and Chile, having in this respect a complementary distribution of that of *T. mexicanus* off California and Baja California.

14. PARALEPIDIDAE
(290 occurrences, 1,648 larvae)

Larvae of Paralepididae were taken in approximately 60 % of the stations occupied on EASTROPAC I. The area of heaviest concentrations was in an equatorial band between lat 5° N and 5° S (Table 16). Two species are

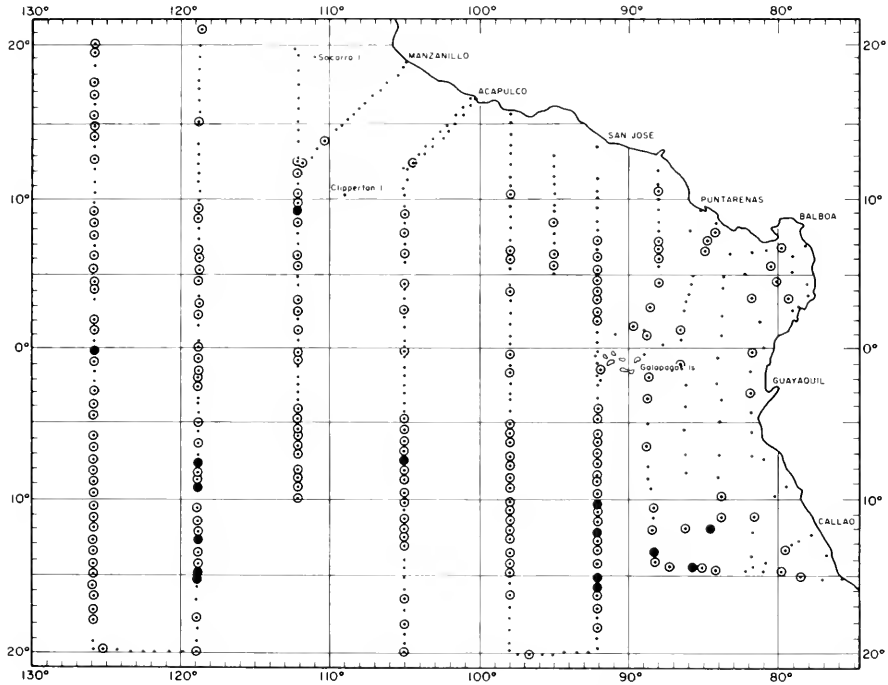

FIGURE 12.—Distribution of larvae of the myctophid, *Symbolophorus evermanni* (Eigenmann and Eigenmann) on EASTROPAC I. Collections of 1 to 25 larvae are shown as large circles with dot in center, collections of 26 or more larvae as large solid circles; negative hauls are shown as small solid circles.

TABLE 16.—Areal occurrence and relative abundance of larvae of Paralepididae on EASTROPAC I.

Latitude	*Argo* 11.000 series		*David Starr Jordan* 12.000 series		*Rockaway* 13.000 series		*Alaminos* 14.000 series		Total EASTROPAC I		
	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae	Average no. larvae per positive haul
20° N-15° N	7	9	15	63	4	19	--	--	26	91	3.5
15° N-10° N	3	6	22	105	14	77	--	--	39	188	4.8
10° N- 5° N	10	38	10	25	8	39	8	21	36	123	3.4
5° N- 0°	12	83	15	100	11	145	21	219	59	547	9.3
0° - 5° S	8	22	18	217	14	136	11	72	51	447	8.8
5° S-10° S	8	36	7	17	8	62	2	11	25	126	5.0
10° S-15° S	13	32	6	24	9	20	2	2	30	78	2.6
15° S-20° S	6	16	4	7	14	25	--	--	24	48	2.0
Total	67	242	97	558	82	523	44	325	290	1,648	5.7

separately tabulated in Appendix Table 3: *Macroparalepis macrurus* Ege (35 occurrences, 44 larvae), and *Sudis atrox* Rofen (13 occurrences, 15 larvae). These two species have such characteristic larvae that they are readily identifiable. The larvae of *Macroparalepis macrurus* were widely distributed in the EASTROPAC area, except in the inner pattern occupied by *Alaminos* (Fig. 7). In contrast, the larvae of *Sudis atrox* were confined to the central water mass of the South Pacific (Fig. 7). This species was originally described from the central water mass of the North Pacific (Rofen, 1963; see also Berry and Perkins, 1966). Preliminary study of the other paralepidid material indicated that a number of species were represented, but that the most common larva was the form illustrated by Ege (1953, Fig. 27), simply as "*Lestidium* spec."

15. EVERMANNELLIDAE
(27 occurrences, 38 larvae)

The larvae of Evermannellidae in the EASTROPAC area have not yet been worked out in detail. Three species of Evermannellidae are known to occur: *Coccorella atrata* (Alcock), *Evermannella indica* Brauer, and a form with a higher anal fin count than is found in these two species. The identity of the latter, known only as yet from larval specimens, remains uncertain. Although larvae of Evermannellidae were not common, the occurrences were distributed over much of the EASTROPAC pattern, except nearshore.

16. SCOPELARCHIDAE
(142 occurrences, 329 larvae)

Scopelarchids are widely distributed in the eastern tropical Pacific, usually occurring in small numbers, i.e., one to three larvae per haul. Only 15 % of the positive hauls contained larger numbers of larvae, i.e. 4 to 20 larvae per haul. Scopelarchid larvae were most common between lat 10° and 20° N, as is shown in Table 17.

There are at least five species of scopelarchids represented by the larvae, and perhaps six. I have not attempted to attach specific names to most of the kinds because the adult scopelarchids

TABLE 17.—Areal occurrence and relative abundance of larvae of Scopelarchidae on EASTROPAC I.

Latitude	*Argo* 11.000 series		*David Starr Jordan* 12.000 series		*Rockaway* 13.000 series		*Alaminos* 14.000 series		Total EASTROPAC I		
	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae	Average no. larvae per positive haul
20° N-10° N	16	38	25	67	15	84	--	--	56	189	3.4
10° N- 0°	4	4	7	12	10	14	13	27	34	57	1.7
0° -10° S	11	15	9	18	5	8	4	6	29	47	1.6
10° S-20° S	4	7	5	7	9	14	5	8	23	36	1.6
Total	35	64	46	104	39	120	22	41	142	329	2.3

of the eastern tropical Pacific are as yet inadequately known. Most larvae taken between lat 10° and 20° N were those of *Scopelarchoides nicholsi* Parr.

17. SCOPELOSAURIDAE
(9 occurrences, 16 larvae)

Two kinds of *Scopelosaurus* larvae were collected in the EASTROPAC area, but neither has been linked to its adult stages as yet; one of these occurred in only a single collection. Most of the specimens of the other form were taken in an equatorial band, between lat 5° N and 5° S.

20. EEL LEPTOCEPHALI
(87 occurrences, 179 larvae in 1.0-m oblique net hauls; 58 occurrences, 553 larvae in 5.0-ft micronekton net hauls)

A total of 10 families of true eels of the order Anguilliformes, suborder Anguilloidei, is represented in the EASTROPAC I collections. Eel leptocephali were decidedly more common in collections made with the 5-ft micronekton net than in the 1-m net collections: 9.5 larvae per positive haul as compared with 2.1 larvae. This difference probably was due in large part to the larger volume of water strained in taking micronekton net hauls, but the faster towing speed of these hauls, ca. 5 knots as compared with 1.5 to 2 knots for 1-m net hauls, also may have contributed. In the discussion of eel families that follows, I have utilized information on occurrence of eel leptocephali from the collections of both nets.

Congridae

Leptocephali of congrid eels were taken at more stations, 57, than those of any other family, yet no congrid larvae were obtained to the south of lat 6° S. Most congrid leptocephali could be identified to genus, of which five were represented; some larvae, however, could not be identified below the family level. Leptocephali of *Ariosoma* were widely distributed between lat 20° N and 3° S, occurring at 28 stations between the coast and the outer line occupied by *Argo*. Leptocephali of *Hildebrandia* were restricted to a broad coastal band, but leptocephali of *Bathyconger* and *Paraconger* were almost as widespread as those of *Ariosoma*. Only one record was obtained of *Gnathopis*.

Derichthyidae

The only definite record is a metamorphosing specimen obtained at station 11.167.

Moringuidae

Leptocephali of *Neoconger* were taken at five coastal stations between lat 8° N and 1° S.

Muraenesocidae

Leptocephali were taken at four stations in the inner pattern occupied by the *Alaminos*, all within 3° of the equator.

Muraenidae

Muraenid leptocephali were taken at 17 stations; two were on the line of stations occupied off Acapulco, Mexico, and the remainder in the broad corridor between Puntarenas, the Galápagos Islands, and the coast of Ecuador.

Nemichthyidae

Two genera of nemichthyid larvae were represented in the EASTROPAC area, *Nemichthys* and *Borodinula*. A specimen of *Nemichthys*, 310 mm long, was obtained at station 14.188. Leptocephali of this family were taken at 24 stations scattered throughout the EASTROPAC area, including the South Pacific central water mass.

Nettastomidae

Taken at 17 stations in the inner half of the EASTROPAC pattern between lat 9° N and 2° S; two kinds of nettastomid larvae were obtained,

one of which was represented by a single specimen.

Ophichthidae

The 31 occurrences of ophichthid eels were distributed in a broad coastal band between Manzanillo, Mexico, and northern Peru (lat 7° S).

Serrivomeridae

Leptocephali of this family were taken at 33 stations, of which 21 were in the outer pattern occupied by *Argo*. Occurrences were grouped into two broad bands—one centered on lat 5° N, the other located between lat 7° and 20° S in the South Pacific central water mass.

Xenocongridae

The leptocephalus of *Chlopsis* was obtained at 22 stations, most located between Panama Bay and the Galápagos Islands.

21. MELAMPHAIDAE
(298 occurrences, 857 larvae)

Melamphaid fishes are the most important family of berycoid fishes in the mesopelagic zone. Four of the five recognized genera occur in the EASTROPAC area: *Melamphaes*, *Scopelogadus*, *Scopelobryx*, and *Poromitra*. According to Ebeling (1962) five species of *Melamphaes* are common in the eastern tropical Pacific, two additional species were collected within the EASTROPAC area, and four other species were collected on the fringes of the area. Only one kind of *Scopelogadus*, *S. mizolepis bispinosus* (Gilbert), is known from the eastern Pacific (Ebeling and Weed, 1963). The remaining two genera, *Scopeloberyx* and *Poromitra*, await revision; the species composition of these genera in the EASTROPAC area is inadequately known. Although melamphaid larvae can be identified to the generic level with some assurance, few developmental series have been worked out at the species level.

Larvae of Melamphaidae were widely distributed in the EASTROPAC area, occurring in 62 % of the collections. Although negative hauls were fewest between the equator and lat 15° N, the average number of larvae per positive haul was rather similar in all areas (Table 18).

23. BREGMACEROTIDAE
(194 occurrences, 1,805 larvae)

Larvae of the gadoid family, Bregmacerotidae, ranked sixth in abundance, contributing 1.9 % of the fish larvae of EASTROPAC I. The only genus, *Bregmaceros*, is widely distributed in pelagic waters of the tropical and subtropical regions of all oceans. D'Ancona and Cavinato (1965) recognized seven species in a worldwide treatment of the genus. These authors stressed the difficulties in species identification.

A preliminary study of EASTROPAC collections of *Bregmaceros* larvae, supplemented by collections of juveniles and adults obtained

TABLE 18.—Areal occurrence and relative abundance of larvae of Melamphaidae on EASTROPAC I.

Latitude	*Argo* 11,000 series		*David Starr Jordan* 12,000 series		*Rockaway* 13,000 series		*Alaminos* 14,000 series		Total EASTROPAC I		
	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae	Average no. larvae per positive haul
20°N-15°N	7	17	15	41	3	5	--	--	25	63	2.5
15° N-10° N	13	36	19	41	18	48	--	--	50	125	2.5
10° N- 5° N	14	59	11	26	24	104	9	24	58	213	3.7
5° N- 0°	7	12	11	19	11	36	24	100	53	167	3.2
0° - 5° S	8	12	9	17	11	56	10	41	38	126	3.3
5° S-10° S	9	18	8	17	9	27	10	21	36	83	2.3
10° S-15° S	6	8	2	6	11	29	6	14	25	57	2.3
15° S-20° S	2	3	2	4	9	16	--	--	13	23	1.7
Total	66	165	77	171	96	321	59	200	298	857	2.9

in micronekton hauls, has shown the presence of five kinds. Larvae of *B. bathymaster* Jordan and Bollman had the most limited distribution, being a coastal species, but were taken in the largest numbers. Two species occurred in the central water mass of the South Pacific (*B. japonicus* Tanaka, and perhaps *B. macellandi* Thompson). Another species occurred in the equatorial current system, and a fifth species was widely distributed between lat 7° and 20° N. One or both of the latter may be undescribed.

26. EXOCOETIDAE
(78 occurrences, 189 larvae)

The species composition of flyingfish larvae has not been worked out in detail as yet. Only larvae of the most common species, *Oxyporhamphus micropterus* (Cuvier and Valenciennes) (51 occurrences, 121 larvae) have been separately tabulated (Appendix Table 3). Larvae of *Oxyporhamphus* were taken at a number of stations in a coastal band off Mexico and central America. Offshore occurrences were limited to an equatorial band between lat 5° S and 7° N. Only one occurrence of larvae of this species was obtained to the south of lat 5° S. Exocoetid larvae undoubtedly are undersampled in oblique plankton hauls, both because of their shallow depth distribution and their marked swimming ability. Much more material of exocoetids—eggs, larvae, and juveniles—are present in surface plankton hauls; only a few of these have been sorted as yet from EASTROPAC I.

28. GEMPYLIDAE-TRICHIURIDAE
(103 occurrences, 231 larvae)

The larvae of these two families are grouped together for reasons discussed below. Larvae of four species of gempylids-trichiurids appear to be widely distributed in the eastern Pacific: these are *Nealotus tripes* Johnson (42 occurrences, 82 larvae, Fig. 7), *Gempylus serpens* Cuvier and Valenciennes (40 occurrences, 57 larvae, Fig. 13), *Diplospinus multistriatus* Maul (26 occurrences, 62 larvae, Fig. 14), and *Lepidopus* sp. (7 occurrences, 25 larvae, Fig. 14). Records of the occurrence of these in EASTROPAC hauls also are given in Appendix Table 5, and summarized in Table 19. One or two specimens each were taken of larvae of two or three additional species of gempylids-trichiurids.

Late larval stages already have been described for three of the above species (Voss, 1954; Strasburg, 1964), but early developmental stages have not been described, except for a species of *Lepidopus*. We plan to describe the early stage larvae of all the above species.

The larval series of these four species raise questions about the distribution of genera between these two families, and perhaps, about the need for two families. Larvae of *Diplospinus multistriatus* are quite similar to those of *Gempylus serpens*. This similarity is marked enough to have led Voss (1954) to describe the larvae of *Diplospinus* as those of *Gempylus* (*i.e.* her *Gempylus* A). Her *Gempulus* B larvae are those of *Gempylus serpens*.

TABLE 19.—Summary of occurrences and relative abundance of species of Gempylidae-Trichiuridae in the four vessel patterns occupied on EASTROPAC I.

Species	*Argo* 11.000 series		*David Starr Jordan* 12.000 series		*Rockaway* 13.000 series		*Alaminos* 14.000 series		Total EASTROPAC I	
	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae
Nealotus tripes	6	6	2	7	12	34	22	35	42	82
Gempylus serpens	8	10	15	19	11	18	6	10	40	57
Diplospinus multistriatus	5	10	0	0	9	31	12	21	26	62
Lepidopus sp. *(xantusi)*	0	0	0	0	1	17	6	8	7	25
Other	0	0	2	2	2	3	0	0	4	5
Total	19	26	18	28	31	103	35	74	103	231

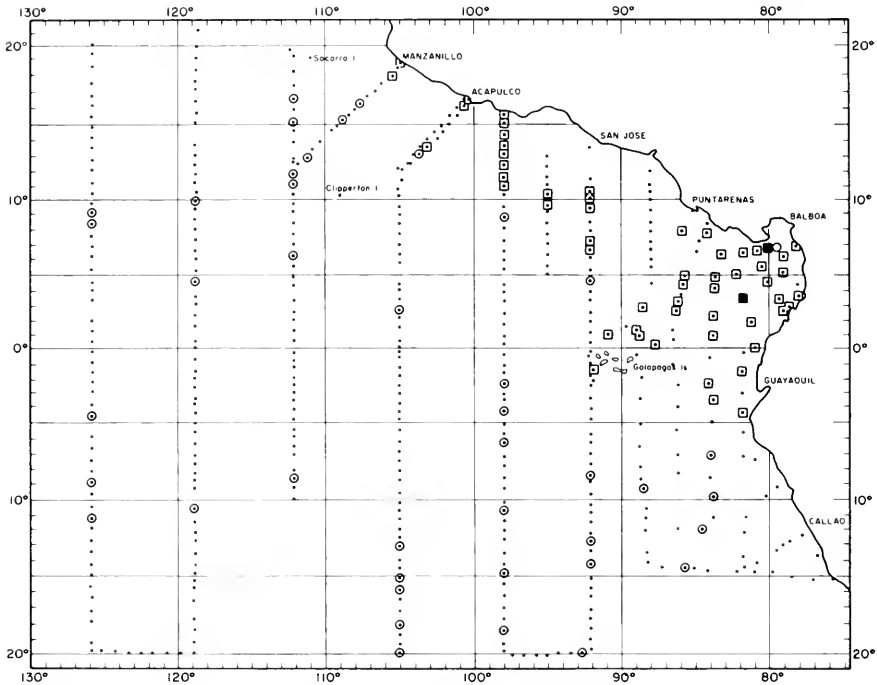

FIGURE 13.—Distribution of larvae of the gempylid, *Gempylus serpens* Cuvier and Valenciennes, and of the cynoglossid flatfish, *Symphurus* spp., on EASTROPAC I. Records of occurrence of larvae of *G. serpens* are shown as large circles with dot in center, *Symphurus* spp. as open squares for hauls containing 1 to 25 larvae and solid squares for hauls with 26 or more larvae; negative hauls are shown as small solid circles.

29. SCOMBRIDAE
(185 occurrences, 1,919 larvae)

Larvae of scombrid fishes ranked fifth in abundance, and made up over 2 % of the larvae. Larvae of the bullet mackerel, *Auxis* spp., (161 occurrences, 1,563 larvae) were by far the most abundant and widely distributed. Larvae of skipjack tuna, *Katsuwonus pelamis* (Linnaeus) (17 occurrences, 214 larvae) were taken mostly in the offshore southern portion of the EASTROPAC area. Other scombrid larvae included yellowfin tuna, *Thunnus albacares* (Bonnaterre) (19 occurrences, 40 larvae); bigeye tuna, *Thunnus obesus* Lowe (1 occurrence, 1 larva); black skipjack, *Euthynnus lineatus* Kishinouye (2 occurrences, 77 larvae); regular *Scomber* sp. (2 occurrences, 7 larvae); Spanish mackerel, *Scomberomorus* sp. (2 occurrences, 3 larvae); and the wahoo, *Acanthocybium solandri* (Cuvier) (1 occurrence, 1 larva). The tuna larvae have been turned over to W. Klawe of the Inter-American Tropical Tuna Commission for detailed study. He kindly has given me permission to include data on occurrence and abundance of larvae of skipjack and bullet mackerel in Ap-

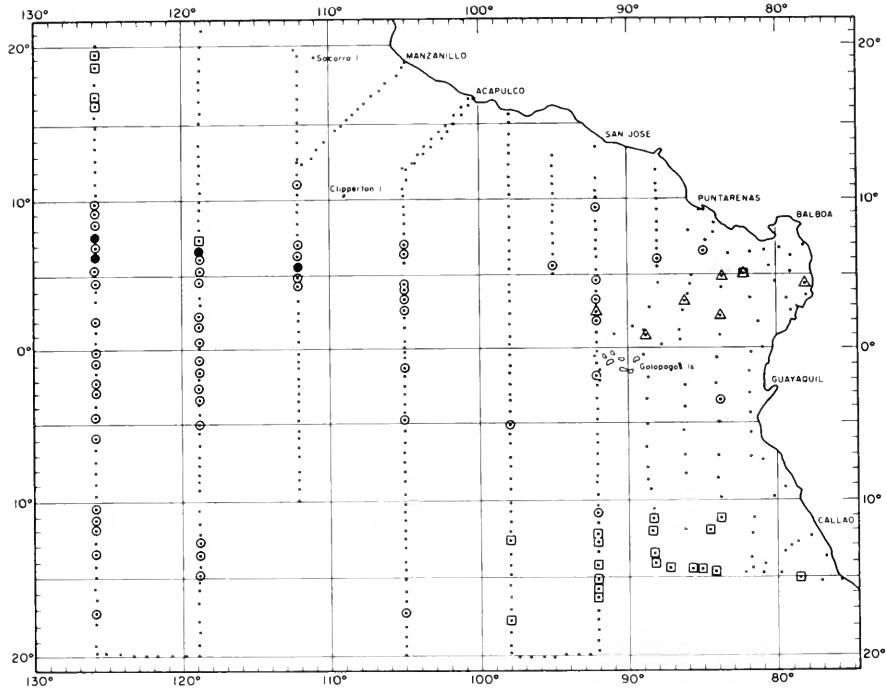

FIGURE 14.—Distribution of larvae of the apogonid, *Howella pammelas* (Heller and Snodgrass), and of the trichiurids, *Diplospinus multistriatus* Maul and *Lepidopus* sp. Records of occurrence of larvae of *H. pammelas* are shown as large circles with dot in center for hauls containing 1 to 10 larvae and large solid circles for hauls containing 11 or more larvae; records of occurrence of larvae of *D. multistriatus* are shown as squares, *Lepidopus* sp. are triangles; negative hauls are shown as small solid circles.

pendix Table 3. Charts showing distribution and relative abundance of larvae of *Auxis* sp. and of *Katsuwonus pelamis* on EASTROPAC cruises will be included in the EASTROPAC Atlas.

30. ISTIOPHORIDAE
(2 occurrences, 2 larvae)

The striking larvae of istiophorids are readily identified to family. The marked paucity of larvae of marlin and sailfish in EASTROPAC I collections was unanticipated, inasmuch as adult billfish are an important part of the Japanese longline catches from the tropical eastern Pacific (Kume and Schaefer, 1966).

32. APOGONIDAE
(61 occurrences, 204 larvae)

Most species of apogonids are coastal, shallow-water forms. A few larvae of these were

taken on EASTROPAC I. However, the majority of apogonid larvae were those of *Howella pammelas* (Heller and Snodgrass), a pelagic species that occurred most commonly in the offshore pattern occupied by *Argo* (Fig. 14). An excellent developmental series has been obtained of this species.

36. CARANGIDAE
(31 occurrences, 183 larvae)

Although a number of kinds of carangid larvae were obtained on EASTROPAC I only larvae of the pilotfish, *Naucrates ductor* (L.), are separately tabulated (Appendix Table 3). Most carangid larvae were taken at stations adjacent to the coast or in the vicinity of offshore islands or banks, and over 50 % of the carangid larvae were obtained at two stations (13.019-70 larvae, 14.016-34 larvae). In these larger collections, the most common carangid larvae were *Chloroscombrus orqueta* Jordan and Gilbert and *Selene brevoorti* (Gill). Several times as many young carangids were taken in one haul of the 5-ft micronekton net as in all plankton samples: 384 specimens at station 14.014. Species composition was as follows: *Naucrates ductor*, 288 specimens, 13.0 to 27.5 mm; *Elagatis bipinnulatus* Quoy and Gaimard, 71 specimens, 18.5 to 42.0 mm; and *Caranx caballus* Günther, 25 specimens, 12.0 to 25.0 mm.

40. CORYPHAENIDAE
(86 occurrences, 118 larvae)

Larvae of the dolphin, *Coryphaena* spp., were widely distributed throughout the EASTROPAC area, but occurred in small numbers, usually one or two specimens per positive haul (average 1.4). The occurrence and abundance of *Coryphaena* larvae in various parts of the EASTROPAC area are summarized in Table 20. The majority of specimens obtained were early-stage larvae; no attempt was made to distinguish between the two species of *Coryphaena*. Charts showing distribution of *Coryphaena* larvae on EASTROPAC cruises will be included in the EASTROPAC Atlas.

44. NOMEIDAE
(178 occurrences, 961 specimens)

The nomeids are an important constituent of the epipelagic fauna of the open ocean. Two genera were represented in the EASTROPAC collections, *Psenes* and *Cubiceps*. Larvae of *Cubiceps* were the more common, but more kinds of *Psenes* larvae were obtained. Altogether, eight different kinds of nomeid larvae have been observed, which differ in meristics, pigmentation, and body shape. In several developmental series of larvae of the genus *Psenes* the pelvic fins developed early, and became conspicuously long and pigmented on older larvae. The larger collections of nomeid larvae were obtained between lat 10° N and 5° S (Fig. 15). Only a few collections were obtained to the south of lat 7° S in the patterns occupied by the *Argo*, *Jordan*, and *Rockaway*, i.e. in the central water mass of the South Pacific. Areal occurrences and relative abundance of nomeid larvae on EASTROPAC I are summarized in Table 21.

TABLE 20.—Areal occurrence and relative abundance of larvae of *Coryphaena* spp. on EASTROPAC I.

Latitude	*Argo* 11.000 series		*David Starr Jordan* 12.000 series		*Rockaway* 13.000 series		*Alaminos* 14.000 series		Total EASTROPAC I		
	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae	Average no. larvae per positive haul
20° N-10° N	3	4	7	9	6	6	--	--	16	19	1.2
10° N- 0°	14	17	9	17	6	6	9	13	38	53	1.4
0° -10° S	5	6	6	9	4	10	5	7	20	32	1.6
10° S-20° S	2	2	1	1	3	3	6	8	12	14	1.2
Total	24	29	23	36	19	25	20	28	86	118	1.4

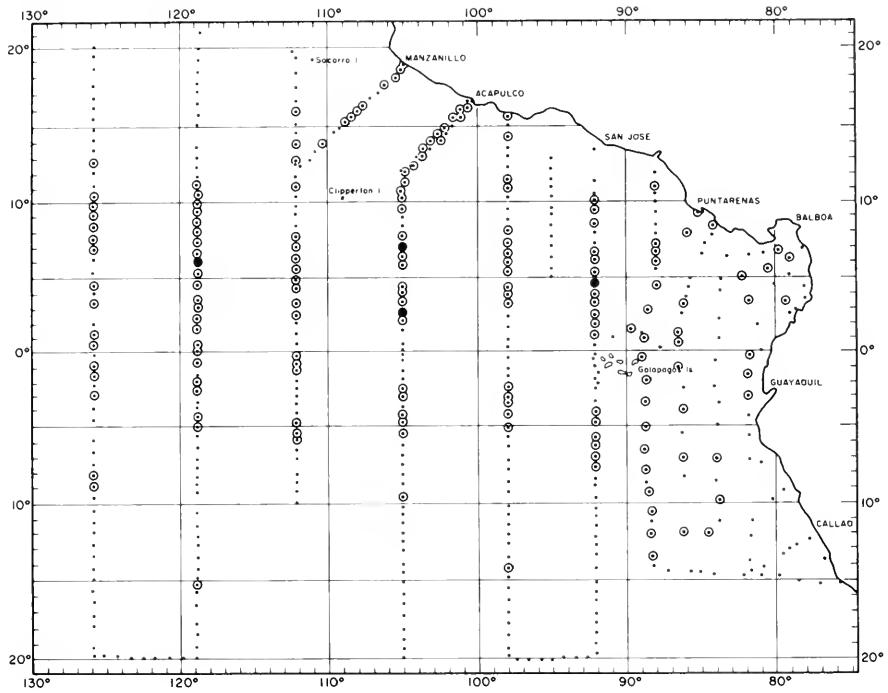

FIGURE 15.—Distribution of larvae of the family Nomeidae on EASTROPAC I. Collections of 1 to 25 larvae are shown as large circles with dot in center, of 26 or more larvae as large solid circles; negative hauls are shown as small solid circles.

TABLE 21.—Areal occurrence and relative abundance of larvae of Nomeidae on EASTROPAC I.

Latitude	*Argo* 11.000 series		*David Starr Jordan* 12.000 series		*Rockaway* 13.000 series		*Alaminos* 14.000 series		Total EASTROPAC I		
	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae	Average no. larvae per positive haul
20° N-15° N	0	0	11	39	3	7	--	--	14	46	3.3
15° N-10° N	5	12	11	24	10	26	--	--	26	62	2.4
10° N- 5° N	12	81	9	87	17	87	7	21	45	276	6.1
5° N- 0°	11	46	9	130	9	76	9	39	38	291	7.7
0° - 5° S	8	26	8	60	6	78	8	30	30	194	6.5
5° S-10° S	2	3	4	6	5	16	7	44	18	69	3.8
10° S-15° S	0	0	0	0	1	1	5	12	6	13	2.2
15° S-20° S	1	10	0	0	0	0	--	--	1	10	10.0
Total	39	178	52	346	51	291	36	146	178	961	5.4

51. TETRAGONURIDAE
(6 occurrences, 7 specimens)

Only a few specimens of *Tetragonurus* larvae were obtained in EASTROPAC I collections. Larvae of *Tetragonurus* have been taken rather commonly in the California Current region and were an important constituent in NORPAC collections. These interesting oceanic fishes were revised by Grey (1955), who recognized three species. Two of these were present in the EASTROPAC area: *T. atlanticus* Lowe and *T. cuvieri* Risso. Late-stage larvae of the two species can be separated by differences in their meristics, and also by differences in pigmentation and body form; larvae of *T. atlanticus* are more heavily and uniformly pigmented and are deeper bodied than larvae of *T. cuvieri* (Grey, 1955).

PLEURONECTIFORMES
(79 occurrences, 503 larvae)

Larvae of flatfishes (Pleuronectiformes) in EASTROPAC collections belonged only to the families Bothidae and Cynoglossidae. Information concerning the kinds and numbers of flatfish larvae taken at each of 79 EASTROPAC I stations is contained in Appendix Table 6; this information is summarized in Table 22.

Flatfish larvae were taken in a broad coastal band, several hundred miles wide, between Manzanillo, Mexico, and northern Peru. The occurrences of some kinds of flatfish larvae and juveniles at considerable distances from shore have been commented upon by a number of workers. Kyle (1913) obtained larvae of *Bothus* from across the North Atlantic and larvae of *Syacium* at considerable distances from shore. Bruun (1937a, 1937b) described bathypelagic occurrences of the bothid flatfish, *Chascanopsetta* and *Monolene*, the latter from off Panama, and of the pleuronectid flatfish, *Poecilopsetta*. Ahlstrom (1965) illustrated the widespread offshore distribution of larvae of *Citharichthys* spp. in the California Current region.

54. BOTHIDAE
(56 occurrences, 199 larvae)

Several kinds of bothid flatfish larvae were taken in 20 or more collections, including larvae of *Bothus leopardinus* (Günther), *Syacium ovale*, and *Citharichthys-Etropus*. Some interesting forms taken less frequently included larvae of *Cyclopsetta* sp., *Engyophrys sancti-laurentii* Jordan and Bollman, and of *Monolene*. A short section will be devoted to each of the above.

Bothus leopardinus (Günther) (28 occurrences, 50 larvae)

Although Norman (1934) lists three species of *Bothus* as occurring in the eastern tropical Pacific—*Bothus mancus* (Broussonet), *B. leop-*

TABLE 22.—Frequency of occurrence and relative abundance of the principal kinds of flatfish larvae, Pleuronectiformes, on EASTROPAC I, summarized by vessel pattern.

Flatfish larvae	*Argo* 11.000 series		*David Starr Jordan* 12.000 series		*Rockaway* 13.000 series		*Alaminos* 14.000 series		Total EASTROPAC I	
	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae	No. positive hauls	No. larvae
BOTHIDAE										
Bothus leopardinus	0	0	1	4	15	32	12	14	28	50
Citharichthys-Etropus	0	0	2	2	6	8	18	40	26	50
Cyclopsetta sp.	0	0	0	0	2	2	1	2	3	4
Engyophrys sancti-laurentii	0	0	0	0	2	3	6	6	8	9
Syacium ovale	0	0	2	8	13	60	9	16	24	84
Other Bothidae	0	0	0	0	1	1	1	1	2	2
Total Bothidae	0	0	3	14	24	106	29	79	56	199
CYNOGLOSSIDAE										
Symphurus spp.	0	0	5	17	21	102	37	185	63	304
Total Pleuronectiformes	0	0	6	31	30	208	43	264	79	503

pardinus (Günther), and *B. constellatus* (Jordan)—he notes that the latter is very doubtfully distinct from *B. leopardinus*. Based on larval material, there appears to be only one common, widely distributed species in the eastern Pacific (Fig. 10), which is referred to *B. leopardinus*. It lacks pigmentation, except for a dorsal and ventral finfold spot near the end of the notochord. This finfold pigment has been observed on a number of species of *Bothus*, hence may be a generic character. *Bothus* larvae are readily separable from other bothid flatfish larvae in the EASTROPAC area by a number of characteristics. Young-stage larvae possess a single elongated anterior dorsal ray, which becomes inconspicuous in older larvae. Older larvae are very deep bodied, usually lack pigmentation and lack head spination. The pelvic fin base on the left side originates mostly anterior to the cleithrum, not posterior as in *Syacium*, *Engyophrys*, *Cyclopsetta*, or *Citharichthys*, and the fin on the ventral midline is much broader based than in these genera. Almost 100 specimens of *Bothus* larvae from the tropical eastern Pacific have been cleared and stained (based in part on EASTROPAC material, in part on previous expeditions). The modal number of vertebrae was 10 + 28 = 38.

Several specimens of flatfish larvae were taken on EASTROPAC I, and on previous expeditions, that had an exceptionally heavy, elongated, single anterior dorsal ray, such as have been described for several genera of bothid flatfish of the subfamily Bothinae. However, the pelvic fins formed behind the cleithrum and the fin on the ventral margin was not much wider based than its recessed partner. These intriguing larvae appear to be those of *Monolene*. Two different kinds have been obtained from the eastern tropical Pacific, one form has 10 + 35 vertebrae, the other has 10 + 28 vertebrae. The latter may be the larva of *Monolene asaedai* (Perkins, 1963).

Cyclopsetta sp. (3 occurrences, 4 specimens)

Larvae of *Cyclopsetta* are more closely related to those of *Syacium* than to other bothid genera. Larvae of both genera develop marked opercular spination as well as a sphenotic spine on either side of the head. *Cyclopsetta* larvae develop 8 to 11 elongated anterior dorsal rays, rather than 5 to 8 as in *Syacium*. *Cyclopsetta* larvae also attain a larger size before transformation; larval specimens as large as 32 mm have been observed in the EASTROPAC area. In late-stage larvae of *Cyclopsetta* the anterior group of dorsal rays is quite elongated, but a more striking feature is the marked development of three rays of the left pelvic fin which may extend almost to the base of the caudal fin. The *Cyclopsetta* larvae have a larger number of vertebrae—usually 10 + 29, as compared to 10 + 25 for larvae of *Syacium ovale* (Günther). Three species of *Cyclopsetta* have been described from the tropical eastern Pacific—*C. querna* (Jordan and Bollman), *C. panamensis* (Steindachner), and *C. maculifera* (Garman), but only *C. querna* has been collected with any frequency as juveniles and adults. The usual count of vertebrae in *C. querna* and *C. panamensis* is 10 + 29; the vertebral count of *C. maculifera* is not known.

Engyrophrys sancti-laurentii Jordan and Bollman (8 occurrences, 9 larvae)

Larvae of *Engyophrys* are about as deep bodied as those of *Bothus*. They possess heavy serrations on the ventral edge of the body both fore and aft of the cleithrum; three small spines also develop on the otic region of the head. The pelvic fins develop immediately posterior to the cleithrum and anterior to the posterior group of ventral serrations. A cleared and stained specimen, 18 mm long, from station 13.040 had 10 + 31 vertebrae, 86 dorsal rays, 71 anal rays, and 17 caudal rays.

Syacium ovale (Günther) (24 occurrences, 84 larvae)

A larval stage of *Syacium* was first illustrated by Kyle (1913) as "*Ancylopsetta* sp." *Syacium* has a distinctive larva with heavy opercular spination, a sphenotic spine on either side of

the head, and 5 to 8 elongated anterior dorsal rays. Larvae of the closely related genus, *Cyclopsetta*, also develop opercular and head spination. The opercular spination is more pronounced in *Syacium*—particularly an antlerlike spine that develops on the posterior border of the preoperculum. The three anterior rays of the left pelvic fin become only moderately elongated in *Syacium* larvae; the rays are of about equal length, firmly joined together by a membrane, and pigmented distally. The full complement of dorsal and anal fin rays usually are laid down before the larvae attain a standard length of 10 mm; the largest specimens studied, ca. 20 mm long, were undergoing metamorphosis.

Citharichthys-Etropus (26 occurrences, 50 larvae)

Before discussing problems in identification of *Citharichthys-Etropus* larvae from the EASTROPAC area, some background information will be given on *Citharichthys* larvae in the CalCOFI region. Illustrations of larvae of three species of *Citharichthys* were given in Ahlstrom (1965). Two species, *Citharichthys sordidus* (Girard) and *C. xanthostigma* Gilbert, develop 2 elongated dorsal rays and also 2 elongated ventral rays on larvae larger than about 5 mm; the other species never develops such rays. Another species that occurs off central and southern Baja California, *C. fragilis* Gilbert, also develops 2 elongated rays on the dorsal and ventral fins.

Two species of *Citharichthys*, *C. gilberti* Jenkins and Evermann, and *C. platophrys* Gilbert, and the widely distributed *Etropus crossotus* Jordan and Gilbert are known to occur in the EASTROPAC area. Three kinds of larvae were taken in EASTROPAC collections referable to *Citharichthys* or *Etropus*. The most common kind developed 3 elongated dorsal rays, a less common form developed 2 elongated dorsal rays, and some specimens lacked elongated rays. The form with 3 elongated dorsal rays is almost certainly referable to *Citharichthys*. Larvae of a common Atlantic species, *C. arctifrons* Goode, develop 3 elongated dorsal rays, confirming the presence of this combination in *Citharichthys* larvae. A cleared and stained specimen from station 13.040 with 3 elongated dorsal rays possessed 10 + 25 vertebrae, 78 dorsal rays, and 59 anal rays. The meristics of the dorsal and anal fins could fit either *C. platophrys* or *C. gilberti*. Yet so little is known of *C. platophrys* that I would hesitate to refer the common *Citharichthys* larvae in EASTROPAC material to this species. A similar problem attends larvae of the form that lacks elongated dorsal rays. Two specimens, 11.5 and 12.0 mm, from station 14.014 each had 88 dorsal and 67 anal rays; vertebrae counts were 10 + 23 and 10 + 24. These counts best fit *E. crossotus*, except that the vertebral counts are low. No material of the form with 2 dorsal rays (undoubtedly a *Citharichthys*) has been cleared and stained for precise meristics. A definite identification has yet to be made on all three kinds of larvae.

55. CYNOGLOSSIDAE
(63 occurrences, 304 larvae)

Only one cynoglossid genus, *Symphurus*, occurs in the eastern Pacific. Five or more kinds of *Symphurus* larvae were obtained in EASTROPAC collections; these were obtained in more collections than larvae of bothid flatfishes (63 as compared with 56), and made up a larger percentage of the total flatfish larvae (ca. 60 %). A moderate number of recently transformed specimens of *Symphurus* were obtained in EASTROPAC collections; in contrast, all specimens of bothid flatfish were pretransformation larvae. The distribution of *Symphurus* larvae in EASTROPAC I is shown in Figure 13.

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ful in many ways; he graciously has permitted me to include station information on occurrence and numbers of larvae of *Auxis* sp. and skipjack tuna. I wish particularly to thank David Kramer and H. Geoffrey Moser for reviewing the manuscript.

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APPENDIX TABLE 1.—Counts of fish larvae, tabulated by family, for all stations occupied on EASTROPAC I.

STATION NUMBER	Bathylagidae	Gonostomatidae	Sternoptychidae	Astronesthidae	Chauliodontidae	Idiacanthidae	Other Stomiatoidei	Myctophidae	Paralepididae	Scopelarchidae	Eel leptocephali	Meliamphaeidae	Bregmacerotidae	Exocoetidae	Scombridae	Gempylidae-Trichiuridae	Nomeidae	Bramidae	Chiasmodontidae	Other identified larvae	Unidentified larvae	Disintegrated larvae	Total fish larvae
11.022	0	10	1	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15
.025	0	52	0	0	0	0	0	11	1	3	0	0	0	0	0	0	0	0	0	0	2	0	69
.027	0	36	0	0	0	4	0	14	1	0	0	0	0	0	0	0	0	0	0	1	0	1	57
.030	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3
.032	1	15	0	0	0	0	0	19	0	0	0	0	2	0	0	0	0	0	0	0	2	1	40
.034	1	88	0	0	0	1	0	35	0	0	0	0	0	0	0	0	0	0	0	0	1	2	128
.036	0	26	0	0	0	0	1	3	1	0	0	0	3	0	0	0	0	0	0	0	0	0	34
.038	4	22	0	0	0	4	0	21	2	0	0	0	6	0	0	0	0	0	0	0	1	0	60
.040	0	20	0	0	0	11	0	55	0	3	0	3	3	0	0	0	0	0	0	4	1	0	100
.044	2	9	0	0	0	20	0	5	1	0	0	2	4	0	0	0	0	0	0	0	3	0	46
.046	3	58	0	0	0	22	0	50	0	4	0	1	2	0	0	0	0	0	1	1	1	3	146
.048	12	41	0	0	0	12	0	20	3	1	0	3	1	0	0	0	0	0	0	0	0	1	94
.050	24	23	0	0	0	3	1	36	0	1	0	2	0	0	0	0	0	0	0	0	1	10	101
.052	15	3	15	0	0	2	0	58	0	0	0	4	2	0	0	0	1	1	0	0	0	1	102
.054	11	8	17	0	0	0	0	159	0	1	0	2	0	0	0	0	3	0	0	0	0	4	205
.056	10	8	0	0	0	0	0	67	0	5	0	0	0	0	0	1	5	0	0	0	5	12	113
.058	10	3	14	0	0	0	0	28	0	0	0	6	2	0	0	0	1	0	0	1	1	10	76
.060	13	9	30	2	1	0	0	72	2	0	0	2	0	0	3	0	5	0	0	2	5	1	147
.062	0	0	6	0	0	0	0	21	2	0	0	5	1	0	0	0	1	0	0	0	1	3	40
.064	0	1	22	0	0	0	2	51	0	0	0	3	3	0	0	1	7	2	0	2	3	2	99
.066	0	2	16	1	0	0	0	63	4	0	0	1	7	3	3	0	4	2	0	15	0	5	126
.068	5	77	49	0	2	0	3	229	4	0	3	6	45	1	2	0	26	1	1	10	9	0	473
.070	1	24	21	0	5	1	0	96	6	0	3	1	25	1	0	0	9	1	0	12	8	9	223
.072	2	73	20	0	6	1	4	178	4	0	1	2	11	0	0	1	16	1	0	12	8	0	340
.076	6	689	21	0	5	4	3	90	0	1	0	2	0	0	4	0	2	0	0	4	7	20	858
.080	7	142	11	0	0	0	6	36	3	0	0	2	1	0	0	0	4	0	0	0	7	0	219
.084	11	361	3	0	1	1	0	131	6	1	0	0	1	0	4	0	3	0	1	2	0	26	552
.088	1	324	3	0	0	0	1	104	3	1	0	0	0	0	0	0	7	0	0	3	0	8	455
.094	0	50	2	0	1	0	0	66	14	0	0	0	0	0	0	0	1	0	0	5	4	4	147
.098	2	107	2	0	0	0	0	907	20	0	0	2	1	0	1	0	4	0	23	12	4	12	1097
11.102	6	33	4	0	0	0	0	99	7	0	0	0	0	0	0	0	1	0	0	12	1	5	168
.106	1	10	2	0	1	0	2	22	0	0	1	0	0	0	0	0	0	2	0	6	0	1	48
.110	8	9	7	0	0	0	0	57	0	1	0	0	0	0	0	0	1	0	0	0	3	1	87
.114	1	57	43	0	0	0	1	243	1	0	0	1	0	0	0	1	3	0	0	5	1	1	358
.118	4	7	6	0	0	0	0	84	0	0	0	3	0	0	0	0	0	1	0	2	2	3	112
.120	1	3	2	0	0	0	0	9	0	0	0	1	0	0	0	0	0	0	1	2	3	0	22
.124	0	25	11	0	0	0	0	66	0	0	0	0	0	0	1	0	5	0	0	0	0	3	111
.128	0	98	6	0	0	0	2	98	4	1	0	1	0	0	0	0	10	1	0	3	4	2	230
.130	0	7	6	0	2	0	1	29	2	0	0	0	0	0	0	0	0	1	0	0	3	0	51
.132	0	8	4	0	1	1	5	16	0	0	0	0	0	0	0	0	0	2	0	1	4	0	42
.134	0	28	0	0	0	0	2	109	4	0	0	1	0	4	171	0	0	0	0	3	8	4	334
.136	0	46	33	0	0	2	2	168	9	2	6	4	0	0	2	0	0	0	0	0	7	1	282
.138	0	8	34	0	0	0	5	21	4	0	0	2	6	0	0	1	0	3	0	2	1	0	87
.140	0	5	9	0	0	0	0	12	0	2	2	0	0	0	0	1	0	0	0	0	1	1	33
.142	0	13	7	0	0	0	0	69	8	1	0	1	1	0	0	0	0	0	1	2	0	7	110
.146	0	22	3	0	0	0	0	17	0	0	0	0	0	0	3	1	0	0	0	0	0	3	49
.148	0	77	2	0	0	0	0	13	1	0	0	1	1	0	0	0	0	0	0	0	2	6	103
.150	0	82	2	0	0	1	2	38	2	0	0	0	0	0	4	0	0	0	0	0	0	10	141
.152	0	138	4	0	0	0	1	115	3	0	0	0	1	0	1	0	0	0	0	4	1	0	268
.154	0	8	4	0	0	2	0	15	1	0	0	2	1	0	0	0	0	0	0	5	0	7	45
.156	0	88	3	0	0	0	2	29	4	0	0	2	2	0	0	0	0	0	0	1	5	21	157
.158	0	40	2	0	1	6	0	103	6	0	0	1	3	0	0	1	0	1	0	4	2	29	199
.159	0	102	2	0	0	1	1	117	9	0	1	2	0	1	0	1	10	4	0	3	5	27	286
.161	0	12	3	0	0	1	0	10	0	0	0	0	2	0	0	0	0	0	0	1	0	2	31

APPENDIX TABLE 1.—Counts of fish larvae, tabulated by family, for all stations occupied on EASTROPAC I.—*Continued.*

STATION NUMBER	Bathylagidae	Gonostomatidae	Sternoptychidae	Astronesthidae	Chauliodontidae	Idiacanthidae	Other Stomiatoidei	Myctophidae	Paralepididae	Scopelarchidae	Eel leptocephali	Melamphaeidae	Bregmacerotidae	Exocoetidae	Scombridae	Gempylidae-Trichiuridae	Nomeidae	Bramidae	Chiasmodontidae	Other identified larvae	Unidentified larvae	Disintegrated larvae	Total fish larvae
11.163	0	8	1	0	0	0	2	2	0	0	0	0	1	0	0	0	0	0	0	0	0	0	14
.167	0	30	0	0	0	0	1	20	1	0	0	0	0	0	1	0	0	0	0	0	3	0	56
.169	0	4	1	0	0	0	0	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	9
.171	0	11	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	13
.173	0	9	0	0	0	0	0	5	0	1	0	0	0	0	0	0	0	0	0	1	0	0	16
.175	0	2	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	1	1	0	0	8
.177	0	5	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	2	0	1	11
.179	0	5	0	0	0	0	0	13	0	0	0	0	0	0	0	0	0	0	0	1	0	0	19
.181	0	13	3	0	0	0	0	13	1	0	0	0	0	0	0	0	0	0	0	1	0	0	31
.183	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	8
.185	0	5	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9
.187	0	22	1	0	0	0	1	24	0	1	0	0	0	1	0	0	0	0	0	1	0	1	52
.189	0	21	1	0	0	0	0	19	0	0	0	0	0	0	0	0	0	1	0	0	0	1	43
.191	0	1	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	4
.195	0	14	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18
.197	0	45	1	0	0	0	1	60	3	0	0	0	1	0	2	0	0	0	0	6	0	5	124
.199	0	6	0	0	0	1	0	9	0	0	0	1	0	0	1	0	0	0	0	3	0	5	26
11.201	0	2	2	0	0	0	0	8	0	0	1	0	1	0	0	0	0	0	0	1	0	0	15
.203	0	24	1	0	0	0	0	17	1	0	0	0	1	0	0	0	0	0	0	2	0	4	50
.205	0	53	1	0	0	1	3	40	2	0	0	0	7	0	0	0	0	0	0	0	0	5	112
.207	0	15	0	0	0	1	0	21	2	0	0	0	0	0	0	0	0	0	0	1	0	7	47
.209	0	7	1	0	0	1	0	12	2	3	0	1	4	0	0	0	0	0	0	2	1	2	36
.211	0	39	5	0	0	0	1	28	1	0	0	1	1	0	0	0	0	0	0	2	6	5	89
.213	0	37	5	0	0	0	3	71	3	2	0	0	3	0	4	1	0	0	0	2	4	1	136
.215	0	5	7	0	0	0	0	44	4	0	0	0	6	0	3	0	0	0	0	2	4	5	80
.217	0	8	7	0	0	0	1	16	0	0	0	1	4	0	0	0	0	1	0	3	0	1	42
.219	0	2	15	0	0	0	0	10	1	0	0	0	6	0	1	1	1	0	0	0	2	1	40
.221	0	56	35	0	0	0	0	74	6	2	3	2	6	0	1	0	2	2	0	1	3	1	194
.223	0	13	32	1	0	0	0	20	0	0	1	3	5	0	0	0	0	0	0	0	0	1	76
.226	0	5	4	0	0	1	0	7	0	1	3	1	3	0	0	0	0	0	0	0	0	0	25
.228	0	3	12	1	0	2	0	16	2	0	2	3	4	0	0	1	0	0	0	1	4	3	54
.234	2	17	19	0	0	0	0	46	1	2	0	1	0	0	0	1	0	0	0	6	5	17	117
.238	0	8	7	0	1	0	1	10	0	0	0	1	0	0	0	0	0	2	0	1	0	13	44
.242	4	50	20	1	1	0	0	95	1	1	0	2	0	1	0	0	2	2	1	5	3	6	195
.246	2	52	6	0	4	0	0	198	1	1	0	0	0	0	0	0	0	1	0	4	3	2	274
.250	1	20	6	0	0	0	0	57	1	0	0	2	0	0	0	0	2	0	0	6	0	5	100
.254	0	20	1	0	0	0	2	149	6	1	0	0	0	2	0	0	2	0	7	2	8	23	223
.258	3	54	6	0	0	0	0	108	9	0	0	2	0	0	0	0	0	0	0	2	3	2	189
.262	2	68	1	0	0	0	3	85	9	0	0	0	0	0	0	0	2	0	0	0	2	3	175
.266	3	33	5	0	0	0	0	38	1	0	0	0	0	0	0	0	5	0	0	0	4	0	89
.270	4	19	8	0	0	0	0	17	0	0	0	0	0	0	7	0	0	0	0	1	6	4	66
.278	13	155	13	0	0	1	0	116	3	0	1	1	4	0	0	0	1	0	0	2	4	3	317
.282	1	27	4	0	1	1	1	82	6	0	5	0	7	0	0	0	0	3	1	3	5	4	151
.285	9	10	34	0	0	1	1	30	5	0	3	1	13	1	0	0	1	0	0	5	3	25	142
.287	1	18	13	0	0	3	2	87	8	1	4	5	13	0	0	0	0	0	0	5	5	7	172
.289	0	17	18	0	0	4	1	131	2	0	3	10	6	0	0	0	0	0	0	25	2	10	229
.291	0	2	19	0	0	1	0	39	0	0	2	6	10	0	0	0	1	0	0	5	0	6	91
.293	7	3	46	0	0	1	1	50	2	0	1	5	5	0	6	0	9	3	1	16	2	8	166
.295	10	15	40	0	0	0	0	130	4	0	0	4	0	0	1	2	12	1	0	7	3	3	232
.297	5	12	3	0	0	0	0	297	4	0	0	1	4	0	3	2	2	0	1	5	1	9	349
.299	27	2	15	0	0	0	0	29	0	0	0	4	0	0	5	0	4	1	0	4	0	2	93
11.301	1	13	0	0	0	6	0	8	0	1	0	2	1	0	2	0	2	1	0	4	0	3	44
.303	12	47	0	0	1	8	0	44	0	0	0	4	1	0	0	0	0	1	0	2	0	7	127
.306	4	64	0	0	0	3	0	40	0	0	0	4	1	0	1	0	0	0	1	1	0	4	123

APPENDIX TABLE 1.—Counts of fish larvae, tabulated by family, for all stations occupied on EASTROPAC I.—*Continued.*

STATION NUMBER	Bathylagidae	Gonostomatidae	Sternoptychidae	Astronesthidae	Chauliodontidae	Idiacanthidae	Other Stomiatoidei	Myctophidae	Paralepididae	Scopelarchidae	Eel leptocephali	Melamphaeidae	Bregmacerotidae	Exocoetidae	Scombridae	Gempylidae-Trichiuridae	Nomeidae	Bramidae	Chiasmodontidae	Other identified larvae	Unidentified larvae	Disintegrated larvae	Total fish larvae
11.308	1	6	2	0	1	0	0	13	0	1	0	2	8	0	0	0	1	0	1	3	0	5	44
.310	4	6	2	0	1	0	0	15	0	1	0	6	10	0	0	0	0	0	1	2	2	3	53
.312	0	62	3	0	0	0	0	26	2	0	0	3	1	0	0	0	0	0	0	2	0	1	100
.314	5	32	2	0	0	1	1	27	0	1	0	1	0	0	0	0	0	1	0	1	1	1	74
.316	0	3	3	0	0	0	1	8	0	0	0	3	2	0	0	0	0	3	0	2	1	0	26
.318	0	3	13	0	0	0	0	34	0	1	0	1	2	0	0	5	0	0	0	1	0	1	61
.320	1	10	5	0	0	2	0	11	1	0	0	0	2	0	0	1	0	0	0	1	0	6	40
.322	0	35	11	0	0	2	1	115	0	10	0	3	1	0	0	0	0	0	0	1	1	6	186
.324	0	21	1	0	1	1	0	13	1	1	0	1	4	0	0	1	0	0	0	0	0	3	48
.326	0	36	3	0	0	1	1	31	0	1	0	1	4	0	0	2	0	0	0	0	0	1	81
.328	0	40	7	0	0	4	1	55	2	3	0	5	2	0	0	0	0	0	0	0	0	3	122
12.002	3	13	0	0	0	21	1	37	1	4	0	2	0	0	0	0	0	0	0	0	0	1	83
.004	3	12	0	0	0	32	3	85	1	2	0	3	3	0	0	1	0	0	0	4	0	3	152
.006	2	15	1	0	1	29	5	33	5	1	0	2	2	0	0	0	0	0	1	24	0	2	123
.008	3	65	0	0	0	17	2	88	1	9	0	0	1	0	0	0	2	0	0	12	0	3	203
.010	8	98	0	0	0	22	0	121	1	1	0	7	2	0	0	0	0	0	0	6	1	2	269
.012	4	24	0	0	0	10	1	31	6	1	0	1	1	0	0	0	0	1	0	8	0	2	90
.014	4	38	0	0	0	8	2	23	5	0	0	4	1	0	0	1	3	0	0	8	0	9	106
.016	8	182	0	0	0	10	4	69	6	4	0	2	1	0	1	0	3	0	0	9	0	2	301
.018	0	199	0	0	0	10	7	137	3	2	0	2	1	1	0	0	1	0	0	7	0	0	370
.020	3	23	0	0	0	5	4	74	4	0	0	4	1	0	0	1	2	0	0	4	1	1	127
.022	0	21	0	0	0	0	1	16	6	0	0	0	1	1	0	0	0	0	0	5	0	5	56
.024	3	242	0	0	0	0	32	97	6	0	0	1	0	0	0	0	0	0	0	13	1	5	400
.026	4	101	0	0	0	1	3	121	6	3	0	4	1	0	0	0	3	0	2	19	0	0	268
.028	0	32	0	0	0	0	3	13	3	0	0	4	0	2	3	0	3	0	0	8	2	0	73
.030	6	12	0	0	0	1	0	24	3	0	0	0	6	0	1	0	14	0	0	7	1	3	78
.032	0	13	0	0	0	0	1	20	2	0	1	1	12	0	7	0	3	0	1	23	0	314	398
.033	3	36	0	0	0	2	4	87	5	1	2	2	533	0	6	0	0	0	0	38	5	4	728
.035	1	73	0	0	0	0	4	23	2	1	0	1	70	5	2	0	4	0	1	18	0	3	208
.037	9	11	0	0	0	3	1	36	3	1	0	0	2	0	3	0	2	0	0	0	0	0	71
.039	4	3	0	0	0	10	0	17	2	3	1	1	5	3	0	0	0	0	0	0	0	1	50
.041	3	22	0	0	0	6	3	108	4	1	2	1	3	3	1	0	0	0	0	8	0	2	167
.043	0	28	0	0	0	16	2	94	22	2	0	1	16	6	1	0	1	0	0	4	0	7	200
.045	0	7	0	0	0	4	0	33	2	2	0	1	5	3	0	0	0	0	0	3	0	3	63
.047	1	4	0	0	0	4	0	61	5	2	0	1	21	4	1	2	1	0	0	3	6	5	121
.049	2	54	0	0	0	6	1	61	2	2	0	2	6	0	2	0	1	0	0	2	0	6	147
.051	0	68	0	0	0	7	1	51	1	0	1	1	2	0	0	0	3	0	0	1	0	2	138
.053	1	18	0	0	0	2	0	6	1	0	0	3	2	0	2	0	2	1	0	1	1	0	40
.055	0	7	0	0	0	1	1	7	4	0	0	2	0	0	0	0	2	0	0	2	0	0	26
.057	2	13	0	0	0	2	0	37	6	0	0	2	0	0	0	0	4	1	0	0	1	0	68
.059	21	78	11	0	0	0	0	99	2	0	2	4	0	0	0	0	1	0	1	4	0	1	224
.061	8	6	16	0	0	1	0	41	0	0	0	0	0	0	0	0	0	0	0	0	0	4	76
.063	8	0	32	0	0	0	0	109	1	0	0	4	0	0	0	0	0	0	0	1	2	3	160
.065	11	47	70	0	1	0	2	614	0	0	0	1	0	0	0	0	1	0	0	5	3	2	757
.067	2	35	57	0	3	0	1	366	1	0	0	2	0	3	19	0	45	0	0	13	0	3	550
.069	5	18	50	0	1	0	2	227	0	2	0	2	1	0	2	0	15	1	0	13	0	4	343
.071	9	18	52	0	4	2	0	71	2	0	0	1	7	0	0	0	4	0	1	5	2	5	183
.075	19	126	74	1	8	0	2	294	3	0	0	1	0	2	0	0	20	0	0	10	7	14	582
.077	13	26	13	0	0	0	0	110	3	0	0	2	2	2	1	0	2	0	1	6	11	8	200
.079	17	48	14	0	0	0	0	129	3	2	0	1	6	2	42	0	3	0	0	7	2	26	302
.081	29	75	46	0	1	0	0	389	4	1	0	0	0	2	31	1	68	0	0	4	0	85	736
.084	14	11	45	0	0	0	0	207	7	0	0	2	0	0	9	0	7	0	0	0	1	8	311
.087	13	16	12	0	0	0	0	64	8	0	0	0	0	0	1	0	0	0	0	1	0	2	117

APPENDIX TABLE 1.—Counts of fish larvae, tabulated by family, for all stations occupied on EASTROPAC I.—*Continued.*

STATION NUMBER	Bathylagidae	Gonostomatidae	Sternoptychidae	Astronesthidae	Chauliodontidae	Idiacanthidae	Other Stomiatoidei	Myctophidae	Paralepididae	Scopelarchidae	Eel leptocephali	Melamphaeidae	Bregmacerotidae	Exocoetidae	Scombridae	Gempylidae-Trichiuridae	Nomeidae	Bramidae	Chiasmodontidae	Other identified larvae	Unidentified larvae	Disintegrated larvae	Total fish larvae
12.090	4	16	1	0	0	0	0	18	5	0	0	0	0	0	0	0	0	0	0	2	0	0	46
.092	0	50	3	0	0	0	0	71	9	0	0	0	0	0	0	0	0	0	0	1	1	16	151
.094	3	133	1	0	0	0	3	377	38	0	0	2	0	0	0	0	0	0	5	0	0	0	562
.097	3	61	4	0	0	0	0	101	16	1	0	0	0	0	0	0	0	0	0	2	0	19	207
12.100	12	14	11	0	0	0	3	56	5	1	0	1	0	0	1	0	0	0	0	6	1	11	122
.103	0	37	23	0	0	0	0	124	3	0	0	0	0	0	0	0	0	0	0	0	1	4	192
.106	26	41	33	0	0	1	0	319	5	0	0	0	0	1	4	0	6	0	0	6	3	7	452
.109	1	23	14	0	0	0	0	65	14	0	0	0	0	0	0	0	18	0	0	3	0	3	141
.112	6	5	17	0	0	0	0	32	1	0	0	0	0	0	0	0	0	0	0	1	1	4	67
.115	17	12	55	0	0	0	0	72	8	0	0	4	0	0	36	2	10	0	0	8	1	10	235
.118	0	159	9	0	0	0	0	107	6	1	0	0	0	2	11	7	18	1	0	7	0	2	330
.120	0	6	57	0	0	0	0	15	3	0	0	2	0	0	3	1	1	0	0	0	0	4	92
.122	0	5	36	0	2	0	0	30	2	0	0	0	0	0	0	0	0	0	0	0	4	3	82
.124	0	8	24	0	0	0	0	27	0	0	0	1	2	0	0	0	0	0	0	1	0	3	66
.126	0	22	17	0	14	0	0	108	0	0	0	1	0	2	0	0	0	0	0	1	1	6	172
.128	0	8	8	0	1	0	0	49	0	0	0	0	0	0	0	0	0	0	0	0	0	0	66
.130	0	0	3	0	2	0	0	12	1	0	0	0	0	0	0	0	0	0	0	0	0	0	18
.132	0	7	8	0	0	0	0	8	0	0	0	0	0	0	0	0	1	0	0	0	0	0	24
.134	0	28	11	0	0	0	1	20	1	0	0	0	0	0	0	0	0	0	0	0	0	1	62
.136	0	16	17	0	0	0	0	19	1	0	0	0	0	0	0	0	0	0	0	0	1	2	56
.138	0	12	6	0	0	0	0	10	3	0	1	0	2	0	0	0	0	0	0	0	0	2	36
.140	0	21	9	0	0	0	1	69	0	0	0	2	0	11	2	0	0	0	0	3	2	0	120
.142	0	110	11	0	1	0	1	84	11	3	2	4	3	1	3	0	0	0	0	0	1	4	241
.144	1	162	37	0	1	0	0	72	7	1	2	0	0	1	0	1	0	0	0	1	3	3	292
.146	0	13	1	0	1	1	0	11	1	1	0	0	0	0	0	0	0	0	0	1	0	0	30
.148	0	6	6	0	0	1	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0	2	26
.150	0	36	0	1	0	0	2	86	1	0	0	0	0	0	17	3	0	0	0	1	4	0	151
.152	0	16	3	0	0	2	0	37	0	0	0	0	1	0	0	1	0	0	0	2	0	7	69
.154	0	11	0	0	0	0	0	12	0	1	0	0	4	0	0	0	0	0	0	0	1	2	31
.156	0	8	0	0	0	0	1	14	1	0	0	0	0	0	0	0	0	0	0	3	0	0	27
.158	0	50	1	0	0	0	0	41	4	1	0	1	0	0	0	1	0	0	0	1	0	0	100
.160	0	19	0	0	0	0	1	19	0	0	0	0	0	1	0	0	0	0	0	0	0	2	42
.162	0	1	0	0	0	0	0	5	0	0	1	0	0	0	0	0	0	0	0	1	0	0	8
.164	0	42	0	0	0	0	1	36	1	0	1	3	0	0	0	0	0	0	0	1	0	0	85
.184	0	49	25	0	0	1	0	45	4	5	0	1	0	0	0	0	0	0	0	1	1	1	133
.186	0	2	20	0	7	0	1	5	1	0	0	0	0	0	0	0	0	0	0	1	0	0	37
.188	0	4	31	0	2	0	1	8	3	0	0	1	2	0	0	1	0	0	0	0	0	3	56
.190	0	11	0	0	0	0	1	6	0	0	0	0	0	0	2	0	0	0	0	0	1	1	22
.192	0	27	7	0	0	0	0	45	3	1	0	1	0	1	0	0	0	0	0	0	2	1	88
.194	0	5	3	0	0	0	0	16	0	1	0	0	2	0	0	0	0	0	0	0	1	2	30
.196	0	5	34	0	10	0	0	22	0	0	0	5	0	0	0	0	3	0	0	3	8	7	97
.198	0	2	44	0	1	0	0	12	0	0	0	5	0	0	0	0	1	0	0	1	0	2	68
12.200	0	6	8	0	0	0	0	36	1	0	0	0	0	1	14	0	5	0	0	1	3	4	79
.203	1	4	38	0	0	0	0	91	2	0	0	0	0	0	1	0	0	0	0	0	0	3	140
.206	4	15	14	0	0	0	1	36	2	0	0	3	0	0	1	0	0	1	0	4	0	2	83
.209	4	6	35	0	0	0	0	70	4	0	0	1	0	0	2	0	0	0	0	6	0	1	129
.212	16	34	21	0	0	0	0	300	8	0	0	1	0	0	5	0	0	0	0	3	6	8	402
.215	7	45	23	0	0	0	0	202	6	0	3	0	0	0	12	0	0	0	0	7	0	3	308
.218	1	22	23	0	0	0	0	127	8	1	0	1	0	0	6	0	1	1	0	12	1	8	212
.221	2	44	13	0	0	0	0	209	24	2	0	2	0	0	0	0	1	0	4	8	4	17	330
.224	0	251	11	0	0	0	3	1089	66	5	0	2	0	0	2	0	1	0	10	13	0	10	1463
.227	1	94	14	0	0	0	3	162	27	0	0	1	1	1	1	0	0	0	1	3	5	22	336
.230	3	5	3	0	0	0	0	49	5	1	0	1	0	0	0	0	0	0	0	8	0	3	78
.233	45	52	24	0	0	0	2	250	10	1	0	2	0	0	0	0	0	0	0	3	0	1	390

APPENDIX TABLE 1.—Counts of fish larvae, tabulated by family, for all stations occupied on EASTROPAC I.—*Continued.*

STATION NUMBER	Bathylagidae	Gonostomatidae	Sternoptychidae	Astronesthidae	Chauliodontidae	Idiacanthidae	Other Stomiatoidei	Myctophidae	Paralepididae	Scopelarchidae	Eel leptocephali	Melamphaeidae	Bregmacerotidae	Exocoetidae	Scombridae	Gempylidae-Trichiuridae	Nomeidae	Bramidae	Chiasmodontidae	Other identified larvae	Unidentified larvae	Disintegrated larvae	Total fish larvae
12.235	25	59	11	0	3	0	4	280	11	0	1	1	0	0	11	0	6	0	0	2	0	5	419
.238	17	39	56	0	4	1	3	225	2	3	0	5	4	0	18	0	10	0	0	0	4	5	396
.240	11	19	70	0	3	0	7	54	2	0	0	1	5	0	24	0	10	0	0	6	1	10	223
.242	1	1	44	0	0	1	2	25	1	0	1	2	4	2	12	0	4	2	0	6	3	5	116
.244	3	14	99	1	3	2	2	105	3	2	0	1	8	1	10	0	14	1	0	29	3	2	303
.246	11	3	42	1	0	0	0	248	3	0	1	4	0	1	8	2	5	0	0	14	2	3	348
.248	1	6	8	0	1	0	1	51	0	0	0	1	1	0	1	0	1	0	0	4	0	2	78
.250	1	8	9	0	0	0	0	54	1	0	0	5	6	0	0	0	1	0	0	1	0	3	89
.252	1	3	5	0	0	1	0	44	2	0	0	1	1	0	0	0	0	0	0	0	0	6	64
.254	1	5	13	0	0	2	0	84	5	0	0	0	1	0	0	0	0	0	0	1	0	1	113
.256	3	1	15	0	0	0	2	23	5	0	0	0	5	0	0	0	0	0	0	1	0	1	56
.258	0	0	26	0	0	0	0	43	0	0	0	0	0	0	0	0	0	0	0	0	0	0	69
.260	2	20	4	0	0	1	0	74	4	2	0	4	0	0	0	1	1	0	0	4	0	3	120
.262	2	102	0	0	0	7	3	161	10	4	0	0	3	0	0	1	0	0	0	2	0	0	295
.264	2	20	0	0	0	29	1	26	10	0	0	3	8	0	0	0	0	0	0	3	2	6	110
.265	3	18	0	0	0	32	1	54	5	7	0	2	17	0	0	0	2	0	0	1	2	1	145
.268	0	105	0	0	0	14	1	156	6	6	0	0	4	0	0	0	5	0	0	12	1	6	316
.270	1	29	0	0	0	17	1	60	6	4	0	2	7	0	0	0	0	0	0	3	1	3	135
.272	4	43	0	0	0	6	2	81	4	1	0	5	11	0	0	1	0	0	0	3	5	2	168
.274	0	40	0	0	0	36	1	85	5	1	0	8	4	0	0	0	1	0	0	1	1	4	187
.276	0	138	0	0	0	6	0	20	0	0	0	0	1	0	0	1	0	0	0	0	0	1	167
.278	1	165	0	0	0	1	2	45	0	0	0	1	3	0	0	0	0	0	0	0	1	0	219
.280	0	22	0	0	0	0	0	5	0	0	0	1	0	0	0	0	0	0	0	0	0	1	29
.282	1	23	0	0	0	0	0	8	0	0	0	1	1	0	0	0	0	0	0	0	1	0	35
.284	0	120	0	0	0	0	2	16	0	0	0	0	0	0	0	0	0	0	0	0	0	1	139
13.001	8	93	108	0	1	8	0	41	0	1	0	6	0	0	1	0	0	0	0	0	1	6	274
.003	14	1165	39	0	2	10	1	385	14	1	0	9	0	0	0	0	2	0	0	11	0	4	1657
.005	54	310	18	0	0	11	3	1075	0	1	0	14	0	0	3	0	1	0	0	7	0	6	1503
.007	9	13	38	0	2	4	3	133	0	0	0	10	0	0	7	0	2	0	0	4	0	0	225
.009	9	10	15	0	1	0	1	494	0	0	0	7	0	0	7	0	0	0	0	1	0	5	550
.011	6	2	15	0	0	1	1	374	0	0	0	0	0	0	0	0	0	0	0	2	0	0	401
.013	18	9	5	0	0	0	0	207	0	0	0	1	1	1	1	0	0	0	0	5	0	3	251
.015	4	1	21	0	0	0	0	479	0	0	0	2	0	0	4	0	0	0	0	2	0	0	513
.017	16	63	20	0	1	0	2	559	0	0	0	3	0	5	8	0	0	0	0	3	5	3	688
.019	9	82	19	0	0	2	0	1219	0	0	9	5	62	10	106	0	2	0	0	275	48	12	1860
.021	4	2	0	0	0	0	0	409	0	0	1	1	176	0	24	0	0	0	0	58	6	9	690
.022	1	0	0	0	0	0	0	0	0	0	0	0	3	0	1	0	0	0	0	0	0	0	5
.028	4	6	0	0	0	0	0	2	0	0	1	0	1	0	1	0	0	1	0	0	0	0	16
.030	7	0	0	0	0	0	0	24	0	0	0	7	2	0	7	0	0	0	0	9	0	0	56
.032	0	24	0	0	0	0	0	44	4	0	0	4	8	0	52	0	4	0	0	36	12	0	188
.034	23	61	4	0	0	0	2	158	0	0	0	4	0	6	43	0	7	1	1	46	8	3	367
.036	8	0	2	0	0	0	0	13	0	0	1	3	0	2	0	0	1	0	0	1	0	0	31
.038	28	0	20	0	0	0	0	142	0	0	0	8	0	0	2	0	0	0	0	2	0	10	212
.040	20	17	65	0	0	0	4	469	0	1	2	2	1	0	20	0	0	0	0	10	0	10	621
.042	11	14	85	0	1	0	0	307	0	0	0	3	0	1	4	0	4	0	0	15	0	3	448
.044	10	14	73	0	0	3	1	109	0	1	0	3	0	0	2	0	1	0	1	1	0	1	220
.046	10	78	95	0	1	5	10	105	11	0	0	6	1	0	1	0	3	0	0	3	2	16	347
.048	29	311	177	0	3	6	5	300	22	0	2	12	1	1	1	3	44	0	0	6	0	15	948
.050	10	41	68	0	0	8	1	133	2	0	0	5	0	0	0	0	5	0	0	1	0	3	277
.052	13	199	73	0	0	3	1	79	24	0	0	6	1	0	0	0	4	0	0	22	4	26	455
.054	14	91	26	0	0	1	4	77	24	0	0	2	2	3	11	17	5	0	1	9	7	8	302
.056	33	513	10	0	0	0	18	144	28	0	0	2	1	0	0	2	7	0	0	15	9	4	786
.058	6	338	9	0	0	0	0	2	83	10	0	0	3	0	0	7	0	3	0	0	0	3	6	470

APPENDIX TABLE 1.—Counts of fish larvae, tabulated by family, for all stations occupied on EASTROPAC I.—*Continued.*

STATION NUMBER	Bathylagidae	Gonostomatidae	Sternoptychidae	Astronesthidae	Chauliodontidae	Idiacanthidae	Other Stomiatoidei	Myctophidae	Paralepididae	Scopelarchidae	Eel leptocephali	Melamphaeidae	Bregmacerotidae	Exocoetidae	Scombridae	Gempylidae-Trichiuridae	Nomeidae	Bramidae	Chiasmodontidae	Other identified larvae	Unidentified larvae	Disintegrated larvae	Total fish larvae
.060	5	26	0	0	0	0	0	59	4	0	0	1	0	0	18	0	0	0	0	2	3	2	120
.062	7	8	1	0	0	0	0	44	5	0	1	0	0	0	1	0	0	0	0	8	5	3	83
.064	15	71	6	0	0	0	2	274	21	0	1	2	0	0	1	0	0	0	1	0	5	7	406
.065	2	72	6	0	0	0	4	31	6	0	0	1	0	0	0	0	0	0	0	1	4	1	128
.067	7	54	3	0	0	0	1	34	6	0	0	1	0	0	0	0	0	0	0	1	0	0	107
.069	37	60	33	0	0	1	6	99	7	0	0	7	0	0	1	0	0	0	0	3	0	3	257
.071	37	572	8	0	1	8	6	318	9	0	0	8	0	1	14	6	3	0	1	13	7	3	1015
.073	42	167	53	0	2	27	1	172	27	0	0	11	0	0	3	7	10	1	1	16	7	8	555
.075	8	21	3	0	1	0	0	39	25	0	0	6	0	0	1	2	1	1	0	1	0	4	113
.077	0	59	38	0	0	0	2	89	14	2	0	1	0	0	5	3	3	0	1	2	2	36	257
.079	0	135	43	0	0	0	0	69	3	3	2	1	0	0	5	0	6	0	0	1	0	0	268
.081	2	164	13	0	0	0	1	16	2	0	1	0	0	0	0	8	4	0	0	1	0	3	215
.083	5	43	4	0	0	0	0	17	1	0	0	6	0	0	0	1	0	0	0	0	0	1	78
.085	0	2	1	0	0	0	0	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20
.087	0	66	6	0	0	1	0	37	0	1	0	0	1	0	0	0	0	0	0	3	0	0	115
.089	0	26	29	0	0	0	0	105	2	0	0	1	0	0	0	0	0	0	2	3	0	15	183
.091	0	11	2	0	0	1	1	49	6	0	0	3	2	0	2	0	0	1	1	3	2	17	101
.093	0	3	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	2	13
.095	6	146	4	1	0	29	3	195	1	0	2	4	7	0	10	7	0	0	6	12	2	4	439
.097	3	103	11	0	0	7	2	205	2	2	0	6	4	0	1	5	0	1	4	9	2	1	368
.099	0	16	7	0	1	0	1	48	0	0	0	1	1	0	0	0	0	0	6	1	1	0	83
13.101	3	11	0	0	1	0	0	45	2	3	0	1	7	0	0	7	0	0	0	4	1	0	85
.103	1	162	6	0	1	3	4	255	5	0	0	5	3	0	0	7	0	0	3	14	3	7	479
.105	0	50	4	0	0	1	1	166	2	0	0	3	1	0	0	2	0	1	4	5	2	0	242
.107	0	1	0	0	0	0	0	13	0	0	0	0	0	0	0	1	0	0	1	0	0	0	16
.109	0	12	0	0	0	0	0	27	0	0	0	0	0	0	0	0	0	0	0	2	0	0	41
.111	0	18	1	0	0	0	0	49	1	0	0	1	0	0	0	0	0	0	0	1	0	0	71
.113	0	30	2	0	0	0	1	72	2	0	0	0	2	1	0	0	0	0	0	2	0	0	112
.115	0	8	0	0	0	0	0	25	1	0	0	0	1	0	0	0	0	0	0	1	0	1	37
.117	0	9	4	0	0	0	4	52	1	2	0	1	3	1	0	0	0	0	0	1	1	2	81
.119	0	36	0	0	0	0	4	86	4	0	0	0	0	0	0	4	0	0	0	0	0	0	134
.121	0	17	3	0	0	0	0	22	1	0	0	0	0	0	0	0	0	0	0	0	0	2	45
.123	0	3	2	0	0	0	0	3	1	1	0	2	0	0	0	0	0	0	2	0	0	0	14
.125	0	1	1	0	0	0	0	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	5
.127	0	20	2	0	0	0	3	39	1	1	0	1	1	0	0	0	0	0	0	0	0	1	69
.129	0	11	1	0	0	0	2	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	28
.131	0	6	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	3	16
.133	0	5	4	0	0	0	0	9	0	0	1	0	0	0	0	0	0	0	0	0	2	0	21
.135	0	56	1	0	0	0	1	78	2	0	0	1	1	0	0	0	0	0	0	0	0	0	140
.137	0	40	1	0	0	0	0	50	1	0	0	0	0	0	0	2	0	0	0	2	0	0	96
.139	0	12	1	0	0	0	0	8	0	0	0	0	0	0	0	1	0	0	0	0	1	1	24
.141	0	4	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12
.143	0	76	2	0	0	0	0	86	1	0	0	1	1	0	0	0	0	0	0	0	6	0	173
.145	0	20	2	0	0	0	2	44	2	0	0	0	2	0	0	0	0	0	0	0	0	0	72
.147	0	17	0	0	0	0	0	33	0	0	0	3	4	0	0	2	0	0	0	3	3	0	65
.149	0	14	6	0	0	0	0	29	0	0	0	2	0	0	0	0	1	0	0	0	0	3	59
.151	0	22	0	0	3	0	1	72	0	1	0	0	0	2	0	0	0	0	0	0	1	1	103
.153	0	103	1	0	1	3	1	394	2	1	1	5	3	0	0	1	0	2	0	17	1	3	539
.155	0	8	4	0	0	0	0	16	0	2	0	2	0	0	0	0	0	0	0	2	4	0	38
.157	0	8	8	0	0	1	0	45	1	0	0	0	1	0	1	0	0	0	0	0	0	2	67
.159	0	12	5	0	0	8	0	53	1	1	0	1	0	0	4	2	0	0	0	2	2	1	92
.161	0	24	6	0	0	0	0	65	3	0	0	0	2	0	0	0	0	0	0	0	0	2	102
.163	0	5	2	0	0	0	0	14	0	0	0	0	1	0	0	0	0	0	0	0	0	0	22
.165	0	4	17	0	0	0	0	24	0	0	0	1	2	0	0	0	0	0	0	0	0	0	48

APPENDIX TABLE 1.—Counts of fish larvae, tabulated by family, for all stations occupied on EASTROPAC I.—*Continued.*

STATION NUMBER	Bathylagidae	Gonostomatidae	Sternoptychidae	Astronesthidae	Chauliodontidae	Idiacanthidae	Other Stomiatoidei	Myctophidae	Paralepididae	Scopelarchidae	Eel leptocephali	Melamphaeidae	Bregmacerotidae	Exocoetidae	Scombridae	Gempylidae-Trichiuridae	Nomeidae	Bramidae	Chiasmodontidae	Other identified larvae	Unidentified larvae	Disintegrated larvae	Total fish larvae
13.167	0	19	11	0	0	0	0	33	0	0	0	0	0	0	0	1	0	0	0	1	0	1	66
.169	0	83	10	0	0	0	0	169	0	0	0	1	0	0	0	0	0	0	0	0	0	1	264
.171	0	18	38	0	0	0	1	104	10	0	0	3	0	0	14	1	0	0	0	2	1	3	195
.173	7	4	10	0	0	3	1	121	2	1	0	3	0	0	6	1	0	0	0	1	0	4	164
.175	22	176	14	0	3	1	0	245	5	0	0	5	0	3	45	1	2	0	0	6	5	2	535
.179	15	111	75	0	0	0	0	133	2	1	0	1	0	1	7	3	4	0	0	2	0	0	355
.183	50	39	39	0	2	3	0	80	13	0	0	15	0	0	7	0	22	1	0	3	2	1	277
.187	43	29	49	0	0	0	3	67	7	0	0	3	0	0	1	2	21	0	1	6	0	4	236
.191	10	148	23	0	0	0	1	126	24	0	0	5	0	1	7	1	18	0	0	8	1	3	376
.195	10	202	2	0	0	0	0	182	2	0	0	0	0	0	0	0	0	1	0	1	0	3	403
.199	5	28	3	0	0	0	0	55	5	0	0	2	0	0	0	0	0	0	1	1	0	2	102
13.203	3	16	2	0	0	0	0	20	2	0	0	0	0	0	0	0	0	0	1	3	0	3	50
.207	11	161	4	0	0	0	0	129	11	0	0	1	0	0	0	0	0	0	0	2	0	1	320
.211	5	36	2	0	0	0	1	68	3	0	0	0	0	0	1	0	0	0	0	0	1	1	118
.215	6	7	5	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	2	27
.219	9	10	14	0	0	0	0	11	0	0	1	0	0	0	0	0	0	0	0	0	0	4	49
.223	2	20	3	0	0	0	0	32	5	0	0	2	0	0	0	0	3	0	0	1	3	1	72
.227	9	38	30	0	0	0	0	121	12	0	1	1	0	4	1	0	3	0	0	0	1	0	221
.231	9	34	35	0	0	0	0	82	0	0	0	1	0	0	0	0	2	0	1	1	0	1	166
.235	9	36	78	0	0	1	1	106	0	2	0	1	0	1	1	1	5	0	0	3	6	7	258
.237	6	47	97	0	1	0	1	189	2	1	0	3	0	0	7	0	7	0	0	3	4	3	371
.239	3	44	58	0	0	0	3	179	0	0	0	1	0	0	0	0	7	0	0	4	0	2	301
.241	2	5	36	0	0	0	0	59	1	0	0	0	0	0	1	0	6	0	0	0	0	4	114
.243	11	13	5	0	0	0	0	93	0	0	0	3	1	0	0	0	5	0	0	1	0	9	141
.245	7	14	11	0	1	0	0	57	3	3	0	2	0	0	0	1	0	0	0	15	0	0	114
.247	9	11	3	0	0	0	0	26	0	2	0	1	0	0	1	0	0	0	0	7	0	0	60
.249	1	3	4	0	0	0	0	46	0	0	0	0	0	0	0	0	0	0	0	1	0	0	55
.251	6	10	9	0	0	1	2	25	15	2	3	1	0	0	3	0	1	1	0	1	0	1	81
.253	20	22	2	0	0	2	4	6	2	0	2	2	0	0	0	0	6	0	0	80	0	0	148
.255	7	29	0	0	0	2	0	29	2	2	0	3	4	1	15	0	0	0	0	29	3	2	128
.257	8	3	0	0	0	0	2	10	3	0	0	1	5	0	0	0	0	0	0	2	0	4	38
.259	17	18	0	0	0	4	0	23	0	0	0	1	2	0	5	0	0	0	0	3	0	6	79
.261	8	37	0	0	0	3	0	13	6	3	0	1	5	0	0	0	1	0	0	44	0	1	122
.263	19	54	0	0	0	4	0	46	1	5	0	3	3	1	11	0	0	0	0	15	1	2	165
.265	6	13	0	0	0	0	8	14	6	1	1	0	5	0	2	0	2	0	0	5	5	1	69
.266	0	2	0	0	0	0	0	14	0	0	0	0	22	0	2	0	0	0	0	8	5	1	54
.268	3	26	0	0	0	7	0	33	5	1	0	1	3	0	1	0	3	0	0	2	4	7	96
.270	4	20	0	0	0	5	4	9	7	1	0	1	1	12	1	0	2	0	0	3	2	6	78
.272	2	3	1	0	0	0	2	13	1	0	0	1	1	0	0	0	1	0	0	1	0	2	28
.274	1	52	0	0	0	21	6	63	15	4	1	8	3	3	2	0	7	0	0	9	2	1	198
.276	5	91	0	0	0	13	3	89	10	0	0	1	3	3	20	0	2	0	0	9	1	0	250
.278	1	21	0	0	0	18	2	7	0	3	0	1	2	0	0	0	1	0	0	0	0	1	59
.280	8	8	0	0	0	27	2	31	2	15	0	0	34	0	0	1	0	0	0	6	0	0	134
.282	0	36	0	0	0	9	6	89	5	20	0	0	7	1	0	0	0	0	0	15	1	0	189
.284	18	61	0	0	0	23	3	34	10	15	0	1	13	2	0	0	0	0	0	3	0	2	185
13.318	13	1	0	0	0	5	0	3	0	2	0	2	5	0	1	0	0	0	0	5	0	0	37
.320	8	16	9	0	0	0	0	18	1	4	1	5	2	0	13	0	0	0	0	23	0	0	100
.322	0	5	0	0	0	0	0	0	0	0	1	0	0	2	9	0	0	0	0	6	0	0	23
.324	10	4	1	0	0	3	0	26	1	0	0	0	1	3	4	0	1	0	0	1	0	0	55
.326	13	10	1	0	0	0	0	44	0	4	0	0	2	0	18	0	0	0	0	4	0	0	96
.328	2	9	1	0	0	0	0	35	2	1	0	5	1	1	7	0	23	0	0	4	3	2	96
.330	4	12	8	0	0	0	0	24	0	0	0	0	0	0	0	0	0	0	0	4	0	0	52
.332	11	2	19	0	0	0	0	63	0	0	0	0	0	0	2	0	0	1	0	1	0	1	100

APPENDIX TABLE 1.—Counts of fish larvae, tabulated by family, for all stations occupied on EASTROPAC I.—*Continued.*

STATION NUMBER	Bathylagidae	Gonostomatidae	Sternoptychidae	Astronesthidae	Chauliodontidae	Idiacanthidae	Other Stomiatoidei	Myctophidae	Paralepididae	Scopelarchidae	Eel leptocephali	Melamphaeidae	Bregmacerotidae	Exocoetidae	Scombridae	Gempylidae-Trichiuridae	Nomeidae	Bramidae	Chiasmodontidae	Other identified larvae	Unidentified larvae	Disintegrated larvae	Total fish larvae
13.334	37	21	17	0	0	0	1	116	2	0	0	3	0	0	1	0	1	0	0	5	3	1	208
.338	9	49	26	0	0	0	0	295	0	0	1	0	0	2	5	0	8	0	0	24	0	3	422
.340	4	11	23	0	0	2	0	47	0	0	0	4	0	0	0	0	4	1	0	4	0	0	100
.342	9	24	21	0	0	2	0	76	4	0	1	3	0	0	4	0	0	0	0	1	0	2	147
14.001	39	97	1	1	1	0	6	867	4	5	2	0	194	0	9	1	12	0	0	261	15	195	1710
.006	32	19	38	0	0	0	1	66	2	0	8	0	2	0	10	0	0	0	0	15	25	8	226
.008	34	4	32	0	0	2	1	86	1	0	2	0	1	0	1	0	2	0	0	25	4	2	197
.010	14	19	40	0	0	4	2	198	1	0	2	3	0	0	0	1	0	0	0	12	2	3	301
.012	6	1	7	0	0	1	1	57	1	0	0	3	1	0	0	2	0	0	0	2	4	4	90
.014	42	4	9	0	0	1	0	67	0	0	1	0	9	0	2	0	0	0	0	30	5	28	198
.016	19	1	20	0	0	0	0	8	0	0	0	2	4	0	2	0	0	0	0	44	16	5	121
.017	17	2	16	0	0	1	0	61	1	0	0	2	0	0	0	0	0	0	0	4	0	1	105
.018	41	48	64	0	0	2	2	424	0	0	0	4	0	0	0	0	5	0	0	24	1	19	634
.020	6	10	12	0	0	0	1	229	1	0	0	2	0	0	0	0	0	0	0	10	0	54	325
.022	7	22	14	0	0	0	3	80	0	0	0	5	1	0	0	0	1	0	0	32	4	0	169
.024	6	0	0	0	0	0	0	47	0	0	1	0	0	1	0	0	0	0	0	29	5	22	111
.027	23	31	42	0	0	0	3	387	0	0	2	7	9	0	6	0	0	0	0	87	34	19	650
.029	24	42	25	0	0	0	5	382	0	1	1	2	6	0	1	3	1	0	0	119	47	26	685
.031	30	43	46	0	0	9	2	594	15	0	2	6	0	0	1	1	3	0	0	75	5	43	875
.033	21	5	0	0	0	0	2	26	1	0	0	1	0	0	0	0	0	0	0	9	3	3	71
.040	48	2	0	0	0	0	2	36	8	0	3	4	0	0	6	0	0	0	1	21	3	21	155
.043	65	17	2	0	0	0	1	159	8	0	0	8	1	0	22	0	1	0	3	15	7	4	313
.047	111	3	4	0	0	0	4	22	3	0	0	2	0	3	9	0	6	0	0	7	0	44	218
.051	225	27	1	1	0	0	5	78	3	0	0	1	0	1	46	0	11	0	1	1	3	25	429
.055	154	2	2	0	0	0	0	40	0	0	0	0	0	0	0	0	0	0	0	4	0	8	210
.060	139	0	0	0	0	0	0	18	0	0	1	2	0	0	0	0	0	0	0	54	2	15	231
.066	13	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	11	0	1	26
.069	20	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	97	0	0	119
.076	3	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	11	0	0	15
.078	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.081	2	16	1	0	0	0	0	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	37
.084	2	3	0	0	0	0	0	4	0	0	0	0	0	1	0	0	0	0	0	1	0	2	13
.086	2	0	0	0	1	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	3	13
.088	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
.091	2	40	0	0	0	0	1	43	0	0	0	0	0	0	0	0	0	0	0	3	2	0	91
.095	2	3	0	0	0	0	1	50	0	0	0	0	0	0	0	1	0	0	1	1	0	0	59
.099	2	3	0	0	0	1	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21
14.103	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.106	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	11	0	14
.110	0	8	0	0	0	0	0	27	0	0	0	0	0	0	0	0	0	0	0	1	0	1	37
.112	0	1	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	1	1	5
.114	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
.115	2	6	0	0	1	0	0	5	0	0	0	1	0	0	0	0	0	0	0	2	0	0	17
.117	2	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	5
.118	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.120	1	6	0	0	1	0	0	15	0	0	0	0	0	1	1	0	0	0	0	0	0	0	25
.122	2	11	1	0	0	1	0	19	0	0	0	0	0	0	0	1	0	0	0	1	0	0	36
.123	7	23	0	0	0	7	0	51	0	0	0	1	1	0	0	1	0	0	2	9	0	0	102
.124	7	76	0	0	0	6	0	152	0	0	0	0	4	2	0	3	0	0	2	12	0	4	268
.126	3	20	1	0	2	6	0	53	0	1	0	0	3	0	0	2	0	0	3	4	1	15	114
.127	5	5	0	0	0	0	1	22	0	0	0	0	2	0	0	3	0	0	0	3	0	3	44
.128	5	60	0	0	3	9	0	145	0	0	0	0	5	0	0	6	1	0	1	13	0	0	248
.130	3	44	1	0	1	7	0	45	0	0	0	2	2	0	4	3	2	1	5	3	4	15	142

APPENDIX TABLE 1.—Counts of fish larvae, tabulated by family, for all stations occupied on EASTROPAC I.—*Continued.*

STATION NUMBER	Bathylagidae	Gonostomatidae	Sternoptychidae	Astronesthidae	Chauliodontidae	Idiacanthidae	Other Stomiatoidei	Myctophidae	Paralepididae	Scopelarchidae	Eel leptocephali	Melamphaeidae	Bregmacerotidae	Exocoetidae	Scombridae	Gempylidae-Trichiuridae	Nomeidae	Bramidae	Chiasmodontidae	Other identified larvae	Unidentified larvae	Disintegrated larvae	Total fish larvae
14.131	0	14	0	0	0	0	1	2	0	2	0	0	0	0	0	1	0	0	0	0	2	2	24
.132	8	17	3	0	0	0	0	8	1	2	2	3	0	0	0	0	2	2	0	5	1	0	54
.134	0	4	2	0	0	0	0	11	0	0	2	2	0	0	0	2	2	0	0	1	0	1	27
.136	1	18	3	0	0	0	0	43	0	0	0	0	0	0	0	0	0	0	1	0	0	0	66
.138	9	92	32	0	0	0	0	29	1	1	0	0	0	1	9	3	5	0	1	1	1	4	189
.142	1	53	31	0	0	0	4	110	0	0	0	1	0	0	1	2	5	0	0	1	0	4	213
.146	46	111	9	0	2	0	2	176	10	0	0	3	0	1	8	2	17	1	0	2	0	4	394
.150	34	48	13	0	0	0	1	52	4	0	0	4	0	4	5	1	2	1	3	6	0	87	265
.154	34	382	17	0	0	0	0	92	6	0	0	5	0	2	7	0	1	0	1	20	1	2	570
.158	0	224	2	0	0	0	1	30	10	0	0	0	0	0	3	0	6	0	0	27	5	21	329
.164	8	53	0	0	0	0	2	135	39	0	3	0	0	0	6	1	0	0	1	8	2	26	284
.172	1	97	20	0	0	0	0	26	0	0	2	0	0	0	16	0	0	0	0	43	3	1	209
.174	3	37	12	0	0	0	0	26	7	0	0	1	0	0	0	0	0	0	0	12	7	32	137
.177	0	3	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	77	5	0	86
.183	1	1	0	0	1	0	0	4	1	0	0	0	0	0	0	0	0	0	0	1	0	2	11
.188	34	168	5	0	0	4	1	261	25	1	5	7	0	3	36	1	3	0	0	5	2	0	561
.194	161	129	1	0	0	3	4	1753	40	0	4	23	0	0	22	2	12	0	0	12	7	24	2197
.195	54	17	0	0	0	2	1	252	24	3	0	1	0	0	0	1	0	0	0	6	0	68	429
.199	15	203	20	0	1	2	0	16	13	3	3	5	0	0	20	0	2	1	1	12	5	4	326
14.203	3	2	12	0	0	0	0	179	2	0	0	1	0	0	1	0	2	0	0	4	0	2	208
.209	5	2	5	0	0	0	0	25	0	0	2	0	1	10	0	0	2	0	0	3	0	1	56
.213	34	96	9	0	1	2	2	206	1	2	1	3	0	1	11	0	2	0	0	6	1	0	378
.218	27	39	1	0	0	1	0	177	0	2	0	1	0	2	4	0	1	0	0	3	1	7	266
.220	2	3	6	0	0	0	0	22	9	0	0	1	0	0	13	0	0	0	0	5	4	21	86
.222	2	5	2	0	1	2	2	49	0	2	1	1	0	0	1	1	0	0	0	1	2	1	73
.224	12	37	19	0	0	0	2	115	2	1	1	1	0	0	8	1	0	0	0	12	4	6	221
.228	2	5	10	0	0	1	0	30	3	2	1	2	1	0	2	0	0	0	0	14	3	0	76
.230	1	5	9	0	1	0	1	41	2	0	0	1	0	0	10	0	0	1	0	8	2	8	90
.232	9	49	23	0	0	0	0	31	1	2	0	3	0	0	12	0	0	0	0	8	0	1	139
.234	9	869	1	0	0	3	8	228	10	0	12	1	0	0	185	9	9	0	0	5	1	350	1748
.236	3	16	3	0	0	1	0	34	2	1	1	3	0	0	10	0	0	0	0	8	1	0	83
.240	21	36	18	0	0	2	0	116	16	2	2	5	1	0	85	1	2	0	0	12	3	8	330
.243	24	28	11	0	0	0	0	44	7	0	1	1	0	1	5	0	1	0	0	3	0	2	128
.247	4	52	9	0	0	2	0	86	9	0	0	0	0	0	1	0	1	0	0	2	2	1	169
.251	11	25	6	0	1	1	0	25	8	0	0	4	0	0	1	0	0	0	0	8	0	1	91
.255	210	86	11	0	1	2	0	227	0	1	0	1	0	0	59	0	2	1	0	8	5	17	631
.259	16	40	6	0	0	4	1	53	0	3	1	6	0	0	0	3	0	2	0	7	3	4	149
.263	2	233	4	0	0	0	0	105	0	0	0	1	0	0	0	0	4	0	0	3	0	2	354
.267	3	33	3	0	0	0	1	8	0	0	0	2	0	0	0	0	0	0	0	6	1	0	57
.276	9	58	0	0	0	1	0	13	0	2	0	3	0	0	0	0	3	0	0	3	3	0	95
.280	7	63	0	0	0	4	2	44	1	0	0	0	0	0	0	6	4	0	0	9	4	10	154
.283	3	13	0	0	2	0	0	22	0	1	0	4	0	0	0	1	0	0	1	2	0	1	50
.287	5	62	12	0	1	0	0	15	0	1	0	2	0	0	0	3	10	1	0	10	0	1	123
.291	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.295	4	1	2	0	0	0	0	65	0	0	0	1	0	0	63	1	1	0	0	8	3	1	150
14.300	287	0	5	0	0	0	2	18	0	0	0	0	0	0	1	0	0	0	0	1	0	8	322
.303	493	10	6	0	0	0	3	200	0	0	0	2	0	0	0	0	0	0	0	9	0	0	723
.306	34	2	1	0	0	0	2	32	8	0	0	6	0	0	0	0	0	0	1	1	0	5	92
.310	91	21	18	0	0	0	3	96	6	0	0	8	0	0	0	0	0	0	0	0	0	5	248
.314	24	25	20	0	0	0	2	59	7	0	0	4	0	0	0	0	0	0	0	2	0	1	144
.318	32	70	39	0	0	9	1	566	0	0	0	4	0	0	1	3	0	0	1	93	0	9	828
.323	11	24	37	0	0	0	3	165	0	0	0	2	0	0	0	0	0	0	0	15	0	5	262
.326	27	66	31	0	0	4	4	659	0	0	2	13	1	0	8	1	0	0	0	49	13	33	911
.330	0	1	3	0	0	0	0	23	1	0	0	0	5	0	0	1	0	0	0	1	0	3	38

APPENDIX TABLE 2.—Myctophid larvae, tabulated by genus or species, for all stations occupied on EASTROPAC I.

STATION NUMBER	Benthosema panamense	Ceratoscopelus townsendi	Diaphus spp.	Diogenichthys laternatus	Diogenichthys atlanticus	Electrona sp.	Gonichthys tenuiculus	Hygophum atratum	Hygophum proximum	Lampadena spp.	Lampanyctus spp.	Lepidophanes pyrsobolus	Lobianchia sp.	Loweina rara	Myctophum spp.	Notolychnus valdiviae	Notoscopelus resplendens	Protomyctophum sp.	Symbolophorus evermanni	Triphoturus spp.	Undentified myctophids	Disintegrated myctophids	Total myctophids
11.022	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	4
.025	0	1	1	1	0	0	0	0	4	0	4	0	0	0	0	0	0	0	0	0	0	0	11
.027	0	1	1	4	0	0	0	4	0	0	2	0	0	1	0	0	0	0	0	0	1	0	14
.030	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
.032	0	0	0	6	0	0	0	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19
.034	0	0	2	10	0	0	0	16	0	0	7	0	0	0	0	0	0	0	0	0	0	0	35
.036	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	3
.038	0	0	2	10	0	0	0	4	0	0	1	0	0	0	0	0	0	0	0	0	2	2	21
.040	0	0	5	4	0	0	0	39	0	0	1	0	0	0	0	0	0	0	4	0	2	0	55
.044	0	0	1	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
.046	0	0	4	41	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	1	50
.048	0	0	1	6	0	0	0	0	0	0	13	0	0	0	0	0	0	0	0	0	0	0	20
.050	0	0	0	33	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0	36
.052	0	0	0	56	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	58
.054	0	0	3	147	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	159
.056	0	0	0	56	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	3	67
.058	0	0	0	27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	28
.060	0	0	4	53	0	0	1	0	3	0	9	0	0	0	0	0	0	0	1	0	1	0	72
.062	0	0	0	16	0	0	0	0	1	0	2	0	0	0	0	1	0	0	0	0	1	0	21
.064	0	0	2	43	0	0	0	0	0	0	2	0	0	0	0	3	0	0	0	0	1	0	51
.066	0	0	4	14	0	0	2	0	9	0	4	0	0	0	20	3	0	0	4	1	2	0	63
.068	0	0	67	33	1	0	0	0	14	2	4	7	0	4	21	32	1	0	21	8	0	14	229
.070	0	1	15	8	0	0	0	0	6	0	0	0	0	1	48	6	2	0	4	0	0	5	96
.072	0	0	28	6	0	0	0	0	22	1	12	7	0	0	44	21	2	0	24	7	0	4	178
.076	0	0	0	23	0	0	0	0	0	0	27	0	0	0	0	20	5	0	0	1	1	13	90
.080	0	0	1	18	0	0	0	2	1	0	6	0	0	0	3	2	0	0	1	0	1	1	36
.084	0	0	0	63	0	0	0	0	7	0	13	0	0	0	1	3	0	0	2	0	0	42	131
.088	0	0	2	10	0	0	0	1	9	0	67	0	0	1	7	0	2	0	0	2	0	3	104
.094	0	0	5	8	0	0	0	0	4	0	32	0	0	0	9	1	0	0	0	1	4	2	66
.098	0	0	3	107	0	0	2	50	109	1	404	0	12	1	180	8	10	0	9	1	1	9	907
11.102	0	0	0	21	0	0	0	3	26	0	12	0	0	0	26	0	0	0	1	0	0	10	99
.106	0	0	0	6	0	0	0	0	4	0	0	0	0	0	7	0	0	0	4	0	0	1	22
.110	0	0	0	41	0	6	0	1	1	0	3	0	0	0	7	1	0	0	1	1	0	1	57
.114	0	0	0	182	0	0	0	3	9	0	31	0	0	1	11	1	1	0	2	0	2	0	243
.118	0	0	0	70	0	0	0	1	1	0	9	0	0	0	0	0	0	0	0	0	0	3	84
.120	0	0	0	8	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	9
.124	0	0	0	37	0	0	0	2	5	0	17	0	0	1	0	3	0	0	0	0	0	1	66
.128	0	1	0	31	0	0	0	3	1	0	29	0	0	2	0	6	0	0	16	1	0	8	98
.130	0	1	11	0	0	0	0	0	3	0	4	0	0	0	1	0	0	0	0	0	8	1	29
.132	0	4	3	0	0	0	0	0	1	3	0	0	0	0	1	0	0	0	4	0	0	0	16
.134	0	26	39	0	0	0	0	0	0	0	30	0	0	0	0	2	0	0	0	0	6	6	109
.136	0	30	39	0	0	0	0	0	5	4	5	0	0	0	8	2	0	0	60	0	15	0	168
.138	0	0	10	0	1*	0	0	0	1	2	1	0	0	0	1	0	0	0	2	1	0	2	21
.140	0	2	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	3	0	1	0	12
.142	0	4	6	0	0	0	0	0	1	0	16	0	0	0	0	0	0	0	31	0	2	9	69
.146	0	10	3	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	2	0	0	1	17
.148	0	5	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	4	0	0	3	13
.150	0	0	4	0	0	0	0	0	3	0	3	0	0	0	0	0	0	0	15	0	0	13	38
.152	0	10	13	0	0	0	0	3	19	1	20	0	0	0	1	0	3	0	43	0	2	0	115
.154	0	0	0	0	0	0	0	0	1	0	6	0	0	0	0	0	0	0	5	0	0	3	15

APPENDIX TABLE 2.—Myctophid larvae, tabulated by genus or species, for all stations occupied on EASTROPAC I. —*Continued.*

STATION NUMBER	Benthosema panamense	Ceratoscopelus townsendi	Diaphus spp.	Diogenichthys laternatus	Diogenichthys atlanticus	Electrona sp.	Gonichthys tenuiculus	Hygophum atratum	Hygophum proximum	Lampadena spp.	Lampanyctus spp.	Lepidophanes pyreobolus	Lobianchia sp.	Loweina rara	Myctophum spp.	Notolychnus valdiviae	Notoscopelus resplendens	Protomyctophum sp.	Symbolophorus evermanni	Tripholurus spp.	Unidentified myctophids	Disintegrated myctophids	Total myctophids
11.156	0	4	0	0	0	1	0	0	9	0	9	0	0	0	0	0	0	0	5	0	0	1	29
.158	0	8	14	0	0	2	0	0	23	0	7	0	0	0	2	0	0	0	27	0	1	19	103
.159	0	19	14	0	0	1	0	0	16	0	17	0	0	0	2	1	0	0	25	0	0	22	117
.161	0	3	0	0	0	0	0	0	2	0	2	0	0	0	1	0	0	0	0	0	0	2	10
.163	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
.167	0	1	5	0	0	0	0	0	1	0	6	1	0	0	2	0	0	0	4	0	0	0	20
.169	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	3
.171	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
.173	0	1	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	1	0	1	0	5
.175	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	4
.177	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
.179	0	2	1	0	0	0	0	2	0	0	7	0	0	0	1	0	0	0	0	0	0	0	13
.181	0	3	1	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	3	13
.183	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.185	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	2	0	4
.187	0	4	0	0	0	0	0	4	0	0	11	0	0	0	0	0	0	0	1	0	0	4	24
.189	0	3	5	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	5	19
.191	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	2
.195	0	0	0	0	0	0	0	0	0	0	2	0	0	1	0	0	0	0	1	0	0	0	4
.197	0	14	13	0	0	1	0	0	3	0	7	0	0	0	3	0	0	0	6	0	5	8	60
.199	0	0	0	0	0	0	0	0	0	0	3	0	0	0	1	0	0	0	2	0	0	3	9
11.201	0	1	0	0	0	0	0	0	0	0	1	0	0	0	3	0	0	0	1	0	0	2	8
.203	0	0	4	0	0	0	0	0	0	0	4	0	0	0	4	0	0	0	2	0	1	2	17
.205	0	5	9	0	0	0	0	0	6	0	3	0	0	0	5	0	0	0	8	0	0	4	40
.207	0	7	0	0	0	0	0	0	2	0	1	0	0	0	0	0	0	0	3	0	0	8	21
.209	0	0	1	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	4	0	0	2	12
.211	0	3	0	0	0	0	0	0	13	0	5	0	0	0	0	0	0	0	2	0	0	5	28
.213	0	17	16	0	0	0	0	0	6	0	5	0	0	0	3	0	0	0	17	0	4	3	71
.215	0	4	0	0	0	0	0	0	13	0	8	0	0	0	2	2	0	0	7	0	0	8	44
.217	0	1	2	0	0	0	0	0	5	0	2	0	0	0	0	0	0	0	5	0	0	1	16
.219	0	3	0	0	0	0	0	0	0	0	2	0	0	0	1	0	0	0	3	0	1	0	10
.221	0	0	26	0	0	0	0	0	11	0	4	3	0	0	1	8	0	0	19	0	0	2	74
.223	0	4	0	0	0	0	0	0	3	0	2	0	0	0	2	2	0	0	6	0	1	0	20
.226	0	2	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	2	0	0	1	7
.228	0	0	2	0	0	0	0	0	4	0	0	0	0	0	0	6	1	0	3	0	0	0	16
.234	0	9	1	14	0	0	0	0	7	0	2	0	0	0	1	4	0	0	1	0	0	7	46
.238	0	0	0	3	0	0	0	0	1	0	2	0	0	0	0	0	0	0	2	0	0	2	10
.242	0	0	0	58	0	0	0	2	9	0	13	0	0	1	2	3	0	0	1	2	0	4	95
.246	0	0	1	62	0	0	0	1	14	0	98	0	0	0	16	0	0	0	0	0	0	6	198
.250	0	0	1	17	0	0	0	2	15	0	8	0	0	0	11	1	0	0	0	1	0	1	57
.254	0	1	2	18	0	0	2	1	30	0	4	0	0	0	85	0	0	0	4	0	0	2	149
.258	0	0	0	32	0	0	0	5	1	0	20	0	0	1	0	0	0	0	36	0	11	2	108
.262	0	0	4	57	0	0	0	2	2	0	5	0	0	4	4	2	0	0	0	0	0	5	85
.266	0	1	3	19	0	0	0	0	0	0	1	0	0	1	3	3	0	0	1	1	2	3	38
.270	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	3	0	0	1	1	2	2	17
.278	0	0	9	51	0	1	0	2	3	0	20	0	2	1	7	13	4	0	0	2	0	1	116
.282	0	0	0	14	0	0	0	0	7	0	19	4	0	0	26	3	0	0	8	1	0	0	82
.285	0	0	0	7	0	0	0	0	6	0	0	0	0	0	6	1	0	0	2	0	0	8	30
.287	0	0	17	2	0	0	0	0	14	1	6	2	0	0	16	18	0	0	5	1	2	3	87
.289	0	0	33	17	0	0	0	0	19	8	4	2	0	0	8	12	2	0	14	0	0	12	131

APPENDIX TABLE 2.—Myctophid larvae, tabulated by genus or species, for all stations occupied on EASTROPAC I. —*Continued.*

STATION NUMBER	*Benthosema panamense*	*Ceratoscopelus townsendi*	*Diaphus* spp.	*Diogenichthys laternatus*	*Diogenichthys atlanticus*	*Electrona* sp.	*Gonichthys tenuiculus*	*Hygophum atratum*	*Hygophum proximum*	*Lampadena* spp.	*Lampanyctus* spp.	*Lepidophanes pyrsobolus*	*Lobianchia* sp.	*Loweina rara*	*Myctophum* spp.	*Notolychnus valdiviae*	*Notoscopelus resplendens*	*Protomyctophum* sp.	*Symbolophorus evermanni*	*Triphoturus* spp.	Unidentified myctophids	Disintegrated myctophids	Total myctophids
11.291	0	2	3	13	0	0	0	0	6	0	1	0	0	1	2	3	0	0	0	0	1	7	39
.293	0	0	0	36	0	0	0	0	2	0	2	0	0	1	1	1	0	1	3	0	2	1	50
.295	0	0	7	94	0	0	0	0	4	0	18	0	0	0	0	1	0	1	4	0	0	1	130
.297	0	0	6	263	0	0	0	0	3	0	3	0	0	0	0	0	0	0	1	0	1	20	297
.299	0	1	0	23	0	0	0	0	2	0	2	0	0	0	1	0	0	0	0	0	0	0	29
11.301	0	0	0	5	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	8
.303	0	0	0	42	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	44
.306	0	0	0	37	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	1	40
.308	0	0	0	6	0	0	0	0	0	0	2	0	0	0	0	0	0	0	5	0	0	0	13
.310	0	0	0	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	15
.312	0	0	0	16	0	0	0	1	0	0	1	0	0	0	0	0	0	0	4	0	0	4	26
.314	0	0	2	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	1	1	27
.316	0	0	1	2	0	0	0	0	0	0	1	0	0	0	0	0	0	0	4	0	0	0	8
.318	0	0	0	26	0	0	1	0	2	0	5	0	0	0	0	0	0	0	0	0	0	0	34
.320	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	3	0	0	6	11
.322	0	4	2	21	0	0	0	3	55	0	12	0	0	1	2	0	0	0	1	0	0	14	115
.324	0	2	0	2	0	0	0	0	1	1	4	0	0	0	2	1	0	0	0	0	0	0	13
.326	0	0	1	2	0	0	0	0	3	0	17	0	0	0	1	1	0	0	2	0	2	2	31
.328	0	3	2	3	2	0	0	3	16	1	6	0	0	1	0	5	3	2	6	0	2	0	55
12.002	0	0	7	12	0	0	0	0	0	0	4	0	0	0	0	8	0	0	3	0	2	1	37
.004	0	0	13	69	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	85
.006	0	0	6	22	0	0	0	1	0	0	3	0	0	0	0	0	0	0	0	0	1	0	33
.008	0	0	24	45	0	0	0	10	0	0	8	0	0	0	0	0	0	0	1	0	0	0	88
.010	0	0	18	73	0	0	0	22	0	0	4	0	0	0	0	0	0	0	0	0	0	4	121
.012	0	0	8	12	0	0	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	31
.014	0	0	7	13	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	23
.016	0	0	20	38	0	0	0	4	0	0	7	0	0	0	0	0	0	0	0	0	0	0	69
.018	0	0	60	65	0	0	0	6	0	0	6	0	0	0	0	0	0	0	0	0	0	0	137
.020	0	0	8	60	0	0	0	3	0	0	3	0	0	0	0	0	0	0	0	0	0	0	74
.022	0	0	1	13	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	16
.024	0	0	24	72	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	97
.026	0	0	29	80	0	0	0	9	0	0	2	1	0	0	0	0	0	0	0	0	0	0	121
.028	0	0	6	1	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	2	1	13
.030	0	0	0	22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	24
.032	0	0	10	6	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	3	0	20
.033	63	0	21	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	87
.035	0	0	14	6	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2	23
.037	0	0	22	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	36
.039	0	0	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	17
.041	0	0	107	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	108
.043	0	0	82	2	0	0	3	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	94
.045	0	0	33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33
.047	0	0	48	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	61
.049	0	0	53	7	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	61
.051	0	0	35	13	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	51
.053	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
.055	0	0	1	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7
.057	0	0	2	32	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	37

APPENDIX TABLE 2.—Myctophid larvae, tabulated by genus or species, for all stations occupied on EASTROPAC I. —*Continued.*

STATION NUMBER	Benthosema panamense	Ceratoscopelus townsendi	Diaphus spp.	Diogenichthys laternatus	Diogenichthys atlanticus	Electrona sp.	Gonichthys tenuiculus	Hygophum atratum	Hygophum proximum	Lampadena spp.	Lampanyctus spp.	Lepidophanes pyrsobolus	Lobianchia sp.	Loweina rara	Myctophum spp.	Notolychnus valdiviae	Notoscopelus resplendens	Protomyctophum sp.	Symbolophorus evermanni	Triphoturus spp.	Unidentified myctophids	Disintegrated myctophids	Total myctophids
12.059	0	0	0	26	0	0	5	0	0	0	56	0	0	0	0	7	0	0	0	0	0	5	99
.061	0	0	0	32	0	0	1	0	0	0	5	0	0	0	0	2	0	0	1	0	0	0	41
.063	0	0	0	104	0	0	1	1	0	0	0	0	0	0	0	3	0	0	0	0	0	0	109
.065	0	0	0	555	0	0	3	0	2	0	25	0	0	0	0	2	17	4	2	2	0	1	614
.067	0	0	4	337	0	0	0	0	0	0	17	1	0	0	0	4	0	0	0	0	0	3	366
.069	0	0	0	195	0	0	0	0	2	0	14	0	0	1	0	6	3	1	4	0	1	0	227
.071	0	0	2	25	0	1	0	0	0	0	15	0	2	0	6	11	0	4	0	0	2	3	71
.075	0	0	16	204	0	1	0	0	12	2	11	0	0	0	10	11	3	0	2	3	1	18	294
.077	0	0	6	65	0	0	0	0	7	0	1	0	0	2	4	10	6	0	0	3	0	6	110
.079	0	0	18	80	0	0	0	1	0	0	5	0	0	0	12	10	0	0	0	0	0	3	129
.081	0	3	7	103	0	0	0	0	9	0	37	0	0	0	11	16	2	7	1	0	0	193	389
.084	0	0	27	127	0	0	0	2	2	0	30	0	0	0	1	13	0	3	0	0	1	1	207
.087	0	0	3	38	0	0	0	0	0	0	12	1	0	1	2	2	0	0	0	1	4	0	64
.090	0	0	1	5	0	0	0	0	0	0	7	0	0	0	0	4	0	0	0	0	1	0	18
.092	0	0	0	12	0	0	0	2	12	0	20	0	0	0	6	0	13	0	0	0	5	1	71
.094	0	0	5	62	0	0	13	25	42	0	140	0	0	0	34	35	0	0	3	11	0	7	377
.097	0	0	0	36	0	0	0	3	3	0	27	0	0	0	9	0	3	0	0	0	0	20	101
12.100	0	0	4	33	0	0	1	0	0	0	5	0	0	0	3	0	2	1	0	1	4	2	56
.103	0	0	6	67	0	0	2	2	0	0	35	0	0	0	3	0	1	0	0	5	0	3	124
.106	0	0	10	277	0	0	0	0	0	0	25	0	0	0	0	0	0	1	0	4	2	0	319
.109	0	0	4	41	0	0	0	0	0	0	8	0	0	0	3	0	0	0	0	7	0	2	65
.112	0	0	1	25	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	2	0	3	32
.115	0	0	0	48	0	0	0	0	0	0	17	0	0	0	0	0	0	0	0	2	0	5	72
.118	0	0	12	7	0	0	1	4	0	0	54	0	0	0	2	6	0	0	20	1	0	0	107
.120	0	0	5	1	0	0	0	0	0	0	7	0	0	0	0	0	0	0	2	0	0	0	15
.122	0	8	14	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	6	0	0	0	30
.124	0	8	5	0	0	0	0	1	0	0	6	1	0	1	1	0	0	0	3	0	0	3	29
.126	0	28	19	0	0	0	0	2	2	1	14	0	0	0	3	0	0	0	39	0	0	0	108
.128	0	2	28	0	0	0	0	2	0	0	6	0	0	0	1	1	0	0	8	0	0	1	49
.130	0	5	2	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0	2	0	0	0	12
.132	0	1	2	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	3	0	1	0	8
.134	0	10	2	0	0	0	0	0	1	0	0	2	0	0	0	3	0	0	1	0	0	1	20
.136	0	6	3	0	1	0	1*	0	1	1	3	0	0	0	1	0	1	0	0	0	1	0	19
.138	0	0	1	1	0	0	0	0	1	0	2	0	0	0	2	1	0	0	1	0	0	1	10
.140	0	0	6	0	2	0	0	0	0	0	3	48	0	0	3	3	0	0	3	0	1	0	69
.142	0	19	11	0	2	7	0	1	5	0	4	3	0	0	3	5	2	0	7	9	0	6	84
.144	0	4	21	0	4	14	0	0	10	0	0	0	0	0	2	1	4	0	3	3	2	4	72
.146	0	0	2	0	1	4	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3	0	11
.148	0	0	5	0	1	0	0	0	0	0	0	2	0	0	1	0	0	0	0	0	2	1	11
.150	0	0	47	0	0	0	0	0	1	0	8	13	11	0	0	0	1	0	0	0	0	5	86
.152	0	0	7	0	0	0	1	0	1	0	0	1	22	0	0	0	0	0	0	3	0	2	37
.154	0	3	0	0	0	4	0	0	0	0	2	1	0	0	0	0	0	0	1	0	1	0	12
.156	0	1	2	0	0	0	0	1*	0	1	0	4	2	0	0	1	0	0	0	0	2	0	14
.158	0	7	6	0	0	0	0	2	7	0	5	6	0	0	4	0	0	0	2	0	0	2	41
.160	0	0	1	0	0	0	0	0	5	2	2	3	0	0	2	0	0	0	0	0	0	4	19
.162	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	5
.164	0	11	7	0	0	0	0	0	1	3	0	6	0	0	0	1	1	0	0	2	0	3	1	36
.184	0	1	15	0	0	1	0	0	1	0	10	0	0	0	2	0	0	0	14	1	0	0	45
.186	0	0	2	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	2	0	0	0	5
.188	0	0	3	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	1	0	1	0	8

APPENDIX TABLE 2.—Myctophid larvae, tabulated by genus or species, for all stations occupied on EASTROPAC I. —*Continued.*

STATION NUMBER	*Benthoaema panamense*	*Ceratoscopelus townsendi*	*Diaphus* spp.	*Diogenichthys laternatus*	*Diogenichthys atlanticus*	*Electrona* sp.	*Gonichthys tenuiculus*	*Hygophum stratum*	*Hygophum proximum*	*Lampadena* spp.	*Lampanyctus* spp.	*Lepidophanes pyrsobolus*	*Lobianchia* sp.	*Loweina rara*	*Myctophum* spp.	*Notolychnus valdiviae*	*Notoscopelus resplendens*	*Protomyctophum* sp.	*Symbolophorus evermanni*	*Triphoturus* spp.	Unidentified myctophids	Disintegrated myctophids	Total myctophids
12.190	0	0	2	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	2	0	0	0	6
.192	0	13	1	0	0	0	0	0	2	1	5	4	0	0	1	8	0	0	9	0	1	0	45
.194	0	1	0	0	0	0	0	0	0	0	8	3	0	0	1	1	0	0	1	0	0	1	16
.196	0	2	5	0	0	0	0	0	0	1	9	0	0	1	0	0	0	0	3	0	0	1	22
.198	0	1	1	0	0	0	0	0	0	0	2	0	0	0	0	5	0	0	2	0	1	0	12
12.200	0	0	0	1	0	0	0	1	1	0	13	0	0	0	0	5	0	1	12	0	1	1	36
.203	0	0	0	80	0	0	0	0	0	0	10	0	0	0	0	0	0	0	1	0	0	0	91
.206	0	0	0	10	0	0	0	0	0	0	21	0	0	0	0	0	0	1	0	0	1	3	36
.209	0	0	0	28	0	0	0	1	0	0	35	0	0	0	0	2	0	1	0	1	0	2	70
.212	0	0	13	92	0	0	0	0	0	0	176	0	0	0	1	6	0	5	0	1	0	6	300
.215	0	0	7	92	0	0	0	1	0	0	98	0	0	0	0	0	0	0	0	2	0	2	202
.218	0	0	4	22	0	0	11	4	2	0	61	0	0	0	6	0	12	0	0	0	2	3	127
.221	0	0	2	102	0	0	2	3	13	0	47	0	0	0	18	0	5	0	5	1	0	11	209
.224	0	0	5	315	0	0	5	63	39	0	471	0	1	0	105	6	30	0	6	5	0	38	1089
.227	0	1	6	98	0	0	1	5	4	0	30	0	0	0	10	0	2	0	0	0	1	4	162
.230	0	0	1	22	0	0	1	1	1	0	16	0	0	0	2	3	0	1	1	0	0	0	49
.233	0	0	3	126	0	0	1	0	0	0	107	0	0	2	2	4	0	1	0	1	2	1	250
.235	0	0	0	194	0	0	1	1	0	0	61	0	0	0	3	17	1	1	1	0	0	0	280
.238	0	0	2	145	0	0	0	1	0	0	42	0	0	3	16	11	4	0	1	0	0	0	225
.240	0	0	0	23	0	0	0	1	5	0	5	0	0	1	8	2	3	0	0	0	0	6	54
.242	0	0	0	14	0	0	0	0	3	0	1	0	0	0	1	2	0	1	0	2	1	0	25
.244	0	0	10	17	0	0	0	1	17	2	10	0	0	0	7	15	0	3	12	8	0	3	105
.246	0	0	7	205	0	0	1	0	0	0	16	0	0	0	0	2	2	0	2	4	3	6	248
.248	0	0	0	43	0	0	0	0	0	0	5	0	0	0	0	2	0	0	0	0	0	1	51
.250	0	0	6	38	0	0	1	0	0	0	6	0	0	0	0	2	0	0	0	0	0	1	54
.252	0	0	7	13	0	0	0	0	0	0	12	0	0	0	0	6	0	1	5	0	0	0	44
.254	0	0	9	22	0	0	0	0	0	0	16	0	0	0	0	1	0	1	35	0	0	0	84
.256	0	0	5	11	0	0	0	0	0	0	2	0	0	0	0	0	0	1	3	0	1	0	23
.258	0	0	0	38	0	0	1	0	0	0	1	0	0	0	0	2	0	0	1	0	0	0	43
.260	0	0	0	26	0	0	0	0	0	0	40	0	0	0	2	6	0	0	0	0	0	0	74
.262	0	0	127	24	0	0	0	1	0	0	2	0	0	0	0	0	0	0	7	0	0	0	161
.264	0	1	3	15	0	0	0	0	0	0	3	0	0	0	0	0	0	0	2	0	0	2	26
.265	0	0	17	31	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	4	54
.268	0	0	57	44	0	0	0	54	0	0	1	0	0	0	0	0	0	0	0	0	0	0	156
.270	0	0	12	35	0	0	1	11	0	0	0	0	0	0	0	0	0	0	0	0	0	1	60
.272	0	0	1	61	0	0	0	16	0	0	0	0	0	0	0	0	0	0	0	0	3	0	81
.274	0	1	17	25	0	0	0	40	0	0	0	0	0	0	0	0	0	0	0	0	2	0	85
.276	0	0	7	8	0	0	0	3	0	0	1	0	0	0	0	0	0	0	0	0	0	1	20
.278	0	0	6	19	0	0	0	13	0	0	7	0	0	0	0	0	0	0	0	0	0	0	45
.280	0	0	2	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	5
.282	0	0	0	5	0	0	0	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	8
.284	0	0	0	10	0	0	0	3	0	1	2	0	0	0	0	0	0	0	0	0	0	0	16
13.001	0	0	0	31	0	0	0	0	0	0	6	0	0	0	0	0	1	0	0	1	2	0	41
.003	0	3	0	315	0	0	0	0	0	0	34	0	0	0	0	12	0	0	8	10	3	0	385
.005	0	0	0	1020	0	0	3	0	0	0	21	0	0	0	0	24	2	0	2	1	2	0	1075
.007	0	0	0	115	0	0	1	0	0	0	6	0	0	0	1	7	0	0	2	0	1	0	133
.009	0	0	0	470	0	0	0	0	0	0	21	0	0	0	0	0	1	0	0	0	2	0	494
.011	0	0	0	372	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	374

APPENDIX TABLE 2.—Myctophid larvae, tabulated by genus or species, for all stations occupied on EASTROPAC I. —*Continued.*

STATION NUMBER	Benthosema panamense	Ceratoscopelus townsendi	Diaphus spp.	Diogenichthys laternatus	Diogenichthys atlanticus	Electrona sp.	Gonichthys tenuiculus	Hygophum atratum	Hygophum proximum	Lampadena spp.	Lampanyctus spp.	Lepidophanes pyrsobolus	Loblanchia sp.	Loweina rara	Myctophum spp.	Notolychnus valdiviae	Notoscopelus resplendens	Protomyctophum sp.	Symbolophorus evermanni	Triphoturus spp.	Unidentified myctophids	Disintegrated myctophids	Total myctophids
13.013	0	0	0	186	0	0	1	0	0	0	20	0	0	0	0	0	0	0	0	0	0	0	207
.015	0	0	0	477	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	479
.017	0	0	0	550	0	0	2	0	0	0	5	0	0	0	0	1	0	0	1	0	0	0	559
.019	482	0	0	715	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	13	1219
.021	407	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	409
.022	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.028	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
.030	0	0	0	24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24
.032	0	0	0	44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	44
.034	0	0	0	142	0	0	0	0	0	0	16	0	0	0	0	0	0	0	0	0	0	0	158
.036	0	0	0	11	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	13
.038	0	0	0	122	0	0	0	0	0	0	18	0	0	0	0	0	0	0	0	0	0	2	142
.040	0	0	0	408	0	0	3	0	0	0	47	0	0	0	0	1	1	6	1	0	0	2	469
.042	0	0	0	271	0	0	0	0	0	0	25	0	0	1	0	3	0	6	0	0	0	1	307
.044	0	0	0	79	0	0	0	0	1	0	14	0	0	0	2	7	1	3	1	0	1	0	109
.046	0	0	0	44	0	0	1	0	0	0	28	0	1	0	2	8	0	3	7	1	4	6	105
.048	0	0	1	160	0	0	4	0	3	0	76	3	2	0	5	19	0	4	9	10	2	2	300
.050	0	0	1	71	0	0	2	0	1	0	25	7	1	0	2	11	1	2	4	4	1	0	133
.052	0	0	0	42	0	0	0	0	0	0	14	0	1	1	1	5	0	0	4	4	5	2	79
.054	0	0	0	36	0	0	1	0	0	0	20	0	3	0	0	12	0	0	2	0	3	0	77
.056	0	0	3	33	0	0	0	0	8	0	43	0	2	1	5	8	0	0	4	12	21	4	144
.058	0	0	2	50	0	0	3	0	0	0	16	0	0	1	0	1	0	0	0	5	5	0	83
.060	0	0	0	54	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	2	59
.062	0	0	4	22	0	0	1	3	0	0	7	0	0	0	0	0	1	0	0	3	2	1	44
.064	0	0	5	191	0	0	15	2	1	0	18	0	0	0	6	1	21	1	0	10	3	0	274
.065	0	0	1	12	0	0	2	1	1	0	8	0	0	0	1	0	1	1	0	1	0	2	31
.067	0	0	2	24	0	0	2	0	0	0	2	0	0	0	0	0	3	0	0	0	0	1	34
.069	0	0	2	73	0	0	2	0	0	0	10	0	0	1	0	0	5	0	0	2	1	3	99
.071	0	0	0	284	0	0	6	0	0	0	20	0	0	0	0	0	0	0	2	5	0	1	318
.073	0	0	0	122	0	0	3	0	0	0	40	0	0	0	0	0	0	0	2	5	0	0	172
.075	0	0	0	28	0	0	2	0	0	0	5	0	0	0	0	0	0	0	1	0	0	3	39
.077	0	0	0	60	0	0	1	0	0	0	23	0	0	0	0	0	0	0	1	3	0	1	89
.079	0	0	0	50	0	0	1	0	0	0	8	0	0	0	0	1	0	0	7	2	0	0	69
.081	0	0	0	11	0	0	0	0	0	0	3	0	0	0	0	0	0	0	1	1	0	0	16
.083	0	0	0	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	1	17
.085	0	0	0	9	0	0	0	0	0	0	2	0	0	0	0	0	0	0	6	0	0	0	17
.087	0	9	0	0	0	0	0	3	0	0	0	0	0	0	0	4	0	0	18	0	1	2	37
.089	0	25	1	0	0	0	0	2	0	0	3	0	0	0	0	7	0	0	34	0	32	1	105
.091	0	13	12	0	0	0	0	10	0	0	0	0	0	0	1	1	0	0	8	0	0	4	49
.093	0	0	1	0	0	0	0	1	0	0	0	0	0	0	2	0	0	0	2	0	0	2	8
.095	0	45	11	1	2*	0	0	45	3	0	7	0	0	1	15	21	4	0	30	3	3	4	195
.097	0	20	21	0	18	2	1	33	4	0	3	1	4	0	18	37	7	0	24	6	5	1	205
.099	0	15	4	0	2	0	0	5	2	0	1	0	0	0	0	7	0	0	5	6	1	0	48
13.101	0	6	10	0	1	0	0	6	0	0	0	0	0	0	3	4	0	0	10	5	0	0	45
.103	0	29	46	9	12*	3	0	42	9	0	19	0	4	0	11	16	13	0	26	6	10	0	255
.105	0	30	19	0	6	3	0	22	3	0	5	0	0	0	5	7	0	0	33	24	4	5	166
.107	0	2	1	0	0	0	0	2	1	0	1	0	0	0	2	0	0	0	1	0	2	1	13
.109	0	1	15	0	0	1	0	2	2	0	1	0	0	0	0	1	0	0	1	1	1	1	27
.111	0	6	17	0	0	1	0	5	0	0	3	1	1	0	7	2	1	0	0	4	0	1	49
.113	0	17	26	0	4	1	0	1	0	7	5	1	0	0	1	1	0	0	2	2	2	2	72

APPENDIX TABLE 2.—Myctophid larvae, tabulated by genus or species, for all stations occupied on EASTROPAC I. —*Continued.*

STATION NUMBER	Benthoaema panamense	Ceratoscopelus townsendi	Diaphus spp.	Diogenichthys laternatus	Diogenichthys atlanticus	Electrona sp.	Gonichthys tenuiculus	Hygophum atratum	Hygophum proximum	Lampadena spp.	Lampanyctus spp.	Lepidophanes pyrsobolus	Lobianchia sp.	Loweina rara	Myctophum spp.	Notolychnus valdiviae	Notoscopelus resplendens	Protomyctophum sp.	Symbolophorus evermanni	Triphoturus spp.	Unidentified myctophids	Disintegrated myctophids	Total myctophids
13.115	0	2	4	0	4	2	1*	0	0	2	0	0	0	0	3	1	0	0	0	0	4	2	25
.117	0	2	20	0	0	0	0	0	0	18	7	0	0	1	1	0	0	0	0	0	3	0	52
.119	0	7	56	0	0	2	0	3	0	6	3	1	0	0	0	0	0	0	0	4	4	0	86
.121	0	6	1	0	0	1	1*	4	0	1	2	0	0	0	1	2	0	0	0	0	2	1	22
.123	0	2	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	3
.125	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	2
.127	0	7	3	0	0	3	2*	4	4	3	5	0	1	0	1	4	0	0	0	0	2	0	39
.129	0	7	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	1	0	2	0	14
.131	0	2	1	0	0	2	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	7
.133	0	2	1	0	0	0	0	0	0	2	1	0	0	0	1	0	0	0	0	0	0	2	9
.135	0	12	35	0	0	1	1*	0	1	11	7	0	1	0	0	0	0	0	0	5	2	2	78
.137	0	5	13	0	0	3	0	2	0	2	6	0	0	0	2	6	0	0	0	9	0	2	50
.139	0	0	3	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	1	2	8
.141	0	1	0	0	1	0	0	0	0	0	1	0	0	0	3	1	0	0	0	0	0	1	8
.143	0	15	21	0	6	2	0	4	0	5	7	2	0	0	5	1	0	0	1	13	2	2	86
.145	0	16	6	0	4	0	0	2	0	0	4	0	0	0	0	2	2	0	0	8	0	0	44
.147	0	2	5	0	2	2	0	0	0	0	2	0	0	0	3	2	3	0	1	10	0	1	33
.149	0	2	2	0	2	0	0	1	0	0	1	2	0	0	3	5	0	0	5	3	1	2	29
.151	0	43	6	0	2	0	0	0	0	4	0	3	0	0	2	1	0	0	1	8	1	1	72
.153	0	83	172	0	7	0	0	30	23	0	9	0	0	0	7	26	1	0	11	8	3	14	394
.155	0	4	2	0	0	2	0	0	0	0	0	0	0	0	0	4	0	0	4	0	0	0	16
.157	0	11	19	0	1	1	0	4	0	0	0	0	0	0	0	2	0	0	1	2	0	4	45
.159	0	12	16	0	0	0	0	3	0	0	1	0	0	0	0	7	0	0	7	0	2	5	53
.161	0	27	11	0	1	0	0	4	1	0	4	0	0	0	0	4	0	0	10	0	1	2	65
.163	0	1	2	0	0	0	0	0	0	0	3	0	0	0	0	1	0	0	6	0	0	1	14
.165	0	3	6	0	0	0	0	0	0	0	0	0	1	0	2	3	0	0	8	0	0	1	24
.167	0	16	2	0	0	0	0	2	0	0	3	0	0	0	1	0	0	0	8	0	1	0	33
.169	0	115	1	8	0	0	0	2	0	0	20	1	0	0	0	1	0	0	17	0	0	4	169
.171	0	5	13	54	0	0	1	0	0	0	7	0	0	0	0	2	0	0	8	0	3	11	104
.173	0	2	0	98	0	0	0	0	0	0	13	0	0	1	0	0	0	0	2	0	1	4	121
.175	0	0	1	168	0	0	0	1	0	0	52	0	0	1	2	0	0	0	18	0	2	0	245
.179	0	0	0	122	0	0	1	0	0	0	7	0	0	0	2	0	0	0	0	1	0	0	133
.183	0	0	0	64	0	0	3	0	0	0	7	0	0	1	2	0	0	0	0	2	0	1	80
.187	0	0	0	49	0	0	2	0	0	0	11	0	0	0	0	0	1	0	0	3	0	1	67
.191	0	0	0	78	0	0	2	0	0	0	43	0	0	0	0	0	0	0	0	3	0	0	126
.195	0	0	4	34	0	0	0	0	0	0	125	0	0	2	0	0	0	0	1	16	0	0	182
.199	0	0	1	31	0	0	4	2	0	0	12	0	0	0	3	0	2	0	0	0	0	0	55
13.203	0	0	0	7	0	0	1	3	0	0	3	0	0	0	4	0	0	0	1	0	0	1	20
.207	0	0	1	72	0	0	12	8	1	0	24	0	0	1	2	1	0	0	0	4	0	3	129
.211	0	0	3	40	0	0	5	1	0	0	9	0	0	0	3	1	0	0	0	2	4	0	68
.215	0	0	0	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2	7
.219	0	0	0	6	0	0	1	0	0	0	3	0	0	0	1	0	0	0	0	0	0	0	11
.223	0	0	0	18	0	1	0	0	0	0	9	0	0	0	0	1	0	0	0	2	1	0	32
.227	0	0	2	90	0	0	1	0	3	0	15	0	0	0	0	2	0	0	1	1	6	0	121
.231	0	0	0	29	0	0	2	0	0	0	39	0	0	0	4	3	1	0	0	0	2	2	82
.235	0	0	1	64	0	1	3	0	0	0	14	0	0	1	5	7	0	3	0	1	1	5	106
.237	0	0	0	133	0	0	0	0	0	4	34	0	0	0	0	8	0	1	1	2	1	5	189
.239	0	0	0	131	0	0	1	0	0	0	29	0	0	0	0	7	0	4	2	5	0	0	179
.241	0	0	0	52	0	0	0	0	0	1	1	0	0	0	1	1	0	1	0	0	0	2	59
.243	0	0	0	67	0	0	0	0	0	1	21	0	0	0	0	0	0	0	0	1	0	3	93

APPENDIX TABLE 2.—Myctophid larvae, tabulated by genus or species, for all stations occupied on EASTROPAC I. —*Continued.*

STATION NUMBER	*Benthosema panamense*	*Ceratoscopelus townsendi*	*Diaphus* spp.	*Diogenichthys laternatus*	*Diogenichthys atlanticus*	*Electrona* sp.	*Gonichthys tenuiculus*	*Hygophum atratum*	*Hygophum proximum*	*Lampadena* spp.	*Lampanyctus* spp.	*Lepidophanes pyrsobolus*	*Lobianchia* sp.	*Loweina rara*	*Myctophum* spp.	*Notolychnus valdiviae*	*Notoscopelus resplendens*	*Protomyctophum* sp.	*Symbolophorus evermanni*	*Triphoturus* spp.	Unidentified myctophids	Disintegrated myctophids	Total myctophids
13.245	0	0	0	37	0	0	0	0	0	0	20	0	0	0	0	0	0	0	0	0	0	0	57
.247	0	0	0	17	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	26
.249	0	0	0	43	0	0	0	0	0	0	1	0	0	0	0	0	0	0	2	0	0	0	46
.251	0	0	0	15	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	25
.253	0	0	0	5	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	6
.255	12	0	11	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	2	0	29
.257	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	1	10
.259	0	0	16	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	1	4	23
.261	0	0	8	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	13
.263	0	0	33	5	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	46
.265	0	0	11	2	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	14
.266	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14
.268	0	0	12	19	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33
.270	0	0	5	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	9
.272	0	0	11	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	13
.274	0	0	26	33	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	63
.276	0	0	38	48	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2	89
.278	0	0	2	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	7
.280	0	0	23	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	31
.282	0	0	80	7	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	89
.284	0	0	13	7	0	0	0	0	0	0	13	0	0	0	0	0	0	0	0	0	1	0	34
13.318	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
.320	3	0	11	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18
.322	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.324	0	0	0	25	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	26
.326	0	0	5	39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	44
.328	0	0	0	28	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	35
.330	0	0	0	24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24
.332	0	0	0	62	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	63
.334	0	0	0	111	0	0	1	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	116
.338	0	0	0	274	0	0	0	0	0	0	15	0	0	0	0	1	0	1	0	2	1	1	295
.340	0	0	0	33	0	0	0	0	0	0	5	0	0	0	3	3	0	1	0	1	1	0	47
.342	0	0	0	62	0	0	0	0	0	0	6	0	0	0	2	3	0	0	0	1	1	1	76
14.001	46	0	0	725	0	0	1	0	0	0	49	0	0	0	18	0	0	0	5	17	0	6	867
.006	0	0	9	43	0	0	0	0	0	0	3	0	0	0	1	0	0	0	0	2	8	0	66
.008	0	0	0	78	0	0	0	0	0	0	3	0	0	0	5	0	0	0	0	0	0	0	86
.010	0	0	6	179	0	0	0	2	0	0	4	0	0	0	6	0	0	0	0	0	0	1	198
.012	0	0	0	47	0	0	0	0	0	0	2	0	0	0	7	0	0	0	0	0	0	1	57
.014	0	0	1	65	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	67
.016	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8
.017	0	0	0	54	0	0	1	0	0	0	1	0	0	0	4	0	0	0	0	0	0	1	61
.018	0	0	0	246	0	0	1	0	0	0	148	0	0	0	23	0	0	0	4	1	0	1	424
.020	0	0	0	225	0	0	0	0	0	0	0	0	0	0	2	0	0	0	1	0	1	0	229
.022	0	0	1	73	0	0	0	0	0	0	3	0	0	0	2	0	0	0	1	0	0	0	80
.024	0	0	0	34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13	47
.027	0	0	0	372	0	0	0	0	0	0	11	0	0	0	0	0	0	0	0	0	0	4	387
.029	0	0	5	371	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	382
.031	0	0	4	540	0	0	1	1	0	0	19	0	0	0	13	0	0	0	6	2	1	7	594

APPENDIX TABLE 2.—Myctophid larvae, tabulated by genus or species, for all stations occupied on EASTROPAC I. —*Continued.*

STATION NUMBER	Benthosema panamense	Ceratoscopelus townsendi	Diaphus spp.	Diogenichthys laternatus	Diogenichthys atlanticus	Electrona sp.	Gonichthys tenuiculus	Hygophum atratum	Hygophum proximum	Lampadena spp.	Lampanyctus spp.	Lepidophanes pyrsobolus	Lobianchia sp.	Loweina rara	Myctophum spp.	Notolychnus valdiviae	Notoscopelus resplendens	Protomyctophum sp.	Symbolophorus evermanni	Tripholurus spp.	Unidentified myctophids	Disintegrated myctophids	Total myctophids
14.033	0	0	0	21	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	1	2	26
.040	0	0	1	16	0	0	4	0	0	0	5	0	0	0	9	0	0	0	0	1	0	0	36
.043	0	0	4	25	0	0	9	0	0	0	40	0	0	0	41	0	25	0	2	3	1	9	159
.047	0	0	0	9	0	0	0	0	0	0	4	0	0	0	8	0	0	0	0	1	0	0	22
.051	0	0	0	31	0	0	0	0	0	0	27	0	0	0	12	0	0	0	1	1	0	6	78
.055	0	0	0	37	0	0	1	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	40
.060	0	0	0	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	18
.066	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
.069	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.076	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
.078	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.081	0	0	0	5	0	0	0	0	0	0	8	0	0	0	0	0	0	0	3	1	0	1	18
.084	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
.086	0	0	0	1	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	1	7
.088	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
.091	0	1	0	0	0	0	0	0	0	0	40	0	0	0	0	0	0	0	1	1	0	0	43
.095	0	0	0	17	0	0	0	0	0	0	1	0	0	0	0	0	1	0	19	0	0	12	50
.099	0	0	0	7	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	1	15
14.103	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.106	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.110	0	0	0	6	0	0	0	0	0	0	14	0	0	0	0	0	0	0	0	1	0	6	27
.112	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	2
.114	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2
.115	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	1	0	0	0	5
.117	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
.118	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.120	0	0	1	5	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	4	2	0	15
.122	0	0	0	7	0	0	0	0	0	0	1	0	0	0	0	0	1	0	8	1	0	1	19
.123	0	0	0	7	1	0	1	6	0	0	4	1	0	0	0	1	0	0	16	1	0	13	51
.124	0	6	4	15	1	0	0	40	0	0	18	0	0	1	2	1	2	0	51	1	10	0	152
.126	0	1	3	0	0	0	0	5	0	0	1	0	0	0	5	3	0	0	21	1	0	13	53
.127	0	1	1	0	0	0	1	2	0	0	1	0	0	0	1	0	0	0	5	9	0	1	22
.128	0	3	10	2	0	0	0	33	0	0	5	2	0	0	0	4	3	0	72	9	2	0	145
.130	0	0	1	7	0	0	0	1	0	0	0	3	0	0	5	2	0	0	21	0	0	5	45
.131	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2
.132	0	0	0	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0	3	1	0	1	8
.134	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	11
.136	0	0	0	43	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	43
.138	0	0	0	15	0	0	1	0	0	0	11	0	0	0	0	0	0	0	0	2	0	0	29
.142	0	0	0	108	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	110
.146	0	0	0	152	0	0	5	0	0	0	13	0	0	0	0	0	0	0	0	6	0	0	176
.150	0	0	0	39	0	0	0	0	0	0	6	0	0	0	0	0	0	0	2	0	0	5	52
.154	0	0	2	12	0	0	0	0	0	0	49	0	0	0	16	0	0	0	8	2	3	0	92
.158	0	0	0	17	0	0	0	0	0	0	7	0	0	0	2	0	0	0	0	0	0	4	30
.164	0	0	0	76	0	0	1	0	0	0	30	0	0	0	5	0	1	0	0	2	0	20	135
.172	0	0	0	16	0	0	2	0	0	0	6	0	0	0	0	0	1	0	0	1	0	0	26
.174	0	0	4	11	0	0	1	0	0	0	4	0	0	0	2	3	0	0	1	0	0	0	26
.177	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.183	0	0	0	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	4
.188	0	0	0	181	0	0	3	0	0	0	49	0	0	0	6	2	9	0	5	6	0	0	261

APPENDIX TABLE 2.—Myctophid larvae, tabulated by genus or species, for all stations occupied on EASTROPAC I. —*Continued.*

STATION NUMBER	Benthosema panamense	Ceratoscopelus townsendi	Diaphus spp.	Diogenichthys laternatus	Diogenichthys atlanticus	Electrona sp.	Gonichthys tenuiculus	Hygophum atratum	Hygophum proximum	Lampadena spp.	Lampanyctus spp.	Lepidophanes pyrsobolus	Lobianchia sp.	Loweina rara	Myctophum spp.	Notolychnus valdiviae	Notoscopelus resplendens	Protomyctophum sp.	Symbolophorus evermanni	Triphoturus spp.	Unidentified myctophids	Disintegrated myctophids	Total myctophids
14.194	0	0	0	1551	0	0	7	0	0	0	158	0	0	2	12	4	8	0	5	5	1	0	1753
.195	0	0	0	243	0	0	2	0	0	0	0	0	0	0	3	0	0	0	0	0	0	4	252
.199	0	0	1	10	0	0	0	0	0	0	2	0	0	0	0	1	0	0	2	0	0	0	16
14.203	0	0	0	90	0	0	1	0	0	0	65	0	0	0	3	1	3	0	4	4	1	7	179
.209	0	0	0	23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	25
.213	0	0	0	181	0	0	0	0	0	0	25	0	0	0	0	0	0	0	0	0	0	0	206
.218	0	0	0	168	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	1	0	0	177
.220	0	0	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	22
.220	0	0	0	46	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	49
.224	0	0	0	90	0	0	0	0	0	0	21	0	0	0	0	0	0	0	2	0	0	2	115
.228	0	0	0	28	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	30
.230	0	0	0	39	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	41
.232	0	0	0	21	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	1	0	0	31
.234	0	0	0	98	0	0	0	0	0	0	81	0	0	0	0	0	0	0	0	0	1	48	228
.236	0	0	0	30	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	34
.240	0	0	0	70	0	0	4	0	0	0	12	0	0	1	20	2	2	0	1	1	0	3	116
.243	0	0	0	23	0	0	2	0	0	0	14	0	0	0	2	0	2	0	0	0	0	1	44
.247	0	0	0	41	0	0	3	0	0	0	27	0	0	0	5	0	2	0	1	6	0	1	86
.251	0	0	0	19	0	0	1	0	0	0	3	0	0	0	0	0	0	0	0	1	1	0	25
.255	0	0	1	216	0	0	1	0	0	0	2	0	0	0	3	0	0	0	0	3	1	0	227
.259	0	0	0	46	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	1	3	1	53
.263	0	0	0	94	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	1	0	0	105
.267	0	0	0	4	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	1	0	8
.276	0	0	0	3	0	0	0	0	0	0	1	0	0	0	0	0	0	0	6	0	2	1	13
.280	0	0	0	6	0	0	2	0	0	0	2	0	0	0	0	0	0	0	26	3	0	5	44
.283	0	0	0	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	1	1	0	22
.287	0	0	0	3	0	0	0	0	0	0	4	0	0	0	0	0	0	0	1	2	4	1	15
.291	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.295	0	0	0	49	0	0	0	0	0	0	15	0	0	0	0	0	0	0	0	0	1	0	65
.300	0	0	0	16	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	18
.303	0	0	0	189	0	0	0	0	0	0	5	0	0	0	3	0	0	0	0	3	0	0	200
.306	0	0	0	27	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	4	0	0	32
.310	0	0	1	61	0	0	2	0	0	0	8	0	0	1	5	0	4	0	0	14	0	0	96
.314	0	0	0	38	0	0	0	0	0	0	14	0	0	0	5	0	0	0	0	0	2	0	59
.318	0	0	0	491	0	0	7	0	0	0	38	0	0	0	23	0	5	0	0	2	0	0	566
.323	0	0	8	149	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	3	3	165
.326	0	0	3	573	0	0	0	0	0	0	73	0	0	0	1	0	0	0	0	4	0	5	659
.330	0	0	0	22	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	23

APPENDIX TABLE 3.—Counts of selected categories of fish larvae, tabulated by station, EASTROPAC I.

STATION NUMBER	Bathylagus nigrigenys	Leuroglossus stilbius urotramus	Nansenia spp.	Araiophos sp.	Cyclothone spp.	Diplophos sp.	Ichthyococcus spp.	Maurolicus muelleri	Vinciguerria spp.	Bathophilus filifer	Evermannellidae	Macroparalepis macrurus	Sudis atrox	Scopelosaurus spp.	Oxyporhamphus micropterus	Trachypteridae	Auxis spp.	Katsuwonis pelamis	Coryphaena spp.	Naucrates ductor	Howella pammelas	Tetragonurus sp.	Cerataioidei
11.022	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.025	0	0	0	0	6	0	0	0	46	0	0	1	0	0	0	0	0	0	0	0	0	0	0
.027	0	0	0	0	2	0	0	0	34	0	1	1	0	0	0	0	0	0	0	0	0	0	0
.030	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.032	1	0	0	0	0	0	0	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.034	1	0	0	0	0	0	0	0	88	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.036	0	0	0	0	0	0	0	0	26	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.038	4	0	0	0	0	0	0	0	22	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.040	0	0	0	0	1	1	0	0	18	0	0	0	0	0	0	1	0	0	0	0	0	0	1
.044	2	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.046	3	0	0	0	1	0	0	0	57	0	0	0	0	0	0	1	0	0	0	0	0	0	0
.048	12	0	0	0	0	0	0	0	41	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.050	24	0	0	0	1	0	0	0	22	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.052	15	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.054	11	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.056	10	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.058	10	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	1	0	0	0	0
.060	13	0	0	0	1	0	1	0	7	0	0	1	0	0	0	0	3	0	1	0	0	0	0
.062	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.064	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0
.066	0	0	0	0	0	2	0	0	0	0	0	0	0	0	2	0	2	1	1	0	11	0	1
.068	5	0	0	0	6	1	0	0	70	0	0	1	0	0	0	0	0	0	3	0	3	0	4
.070	1	0	0	0	6	1	0	0	17	0	3	1	0	0	1	0	0	0	0	0	8	0	1
.072	2	0	2	0	6	2	0	0	65	0	0	0	0	0	0	0	0	0	1	0	9	0	1
.076	6	0	0	0	5	0	2	0	682	1	1	0	0	0	0	0	0	4	0	0	0	0	0
.080	7	0	0	0	2	1	0	0	139	3	0	1	0	0	0	0	0	0	0	0	0	0	0
.084	11	0	0	0	3	0	0	0	358	0	0	0	0	0	0	0	3	0	0	0	2	0	0
.088	1	0	0	0	9	0	0	0	315	1	0	1	0	0	0	0	0	0	1	0	2	0	0
.094	0	0	0	0	9	0	0	0	41	0	0	0	0	0	0	1	0	0	1	0	1	0	2
.098	2	0	0	0	20	0	0	0	87	0	0	0	0	0	0	0	0	1	0	0	0	0	1
11.102	6	0	3	0	8	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	2	0	0
.106	1	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	4	0	2
.110	8	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.114	1	0	0	0	11	2	0	0	44	0	0	0	0	0	0	0	0	0	0	0	3	0	1
.118	4	0	0	0	1	2	0	0	4	0	0	0	0	0	0	0	0	0	0	0	2	0	0
.120	1	0	0	0	2	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1
.124	0	0	0	0	7	1	0	0	17	0	0	0	0	0	0	0	1	0	0	0	0	0	0
.128	0	0	0	0	6	0	0	0	92	0	0	0	0	0	0	0	0	0	0	0	2	0	1
.130	0	0	0	0	6	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.132	0	0	0	0	5	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0
.134	0	0	0	0	3	24	0	0	1	0	0	0	0	0	0	1	0	166	0	0	0	2	0
.136	0	0	0	0	24	2	0	0	20	0	0	0	0	0	0	0	0	2	0	0	0	0	0
.138	0	0	1	0	6	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1
.140	0	0	0	0	4	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.142	0	0	0	0	9	0	0	0	4	0	0	0	0	1	0	0	0	0	0	0	0	0	0
.146	0	0	0	20	2	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0
.148	0	0	0	61	15	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.150	0	0	0	70	11	0	0	0	1	0	0	0	0	0	0	0	0	4	0	0	0	0	0
.152	0	0	0	89	38	1	0	0	9	0	0	0	1	0	0	0	0	1	1	0	2	1	0
.154	0	0	2	5	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
.156	0	0	0	83	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.158	0	0	0	20	16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
.159	0	0	0	70	24	0	0	0	6	0	0	0	3	0	0	0	0	0	0	0	0	0	0
.161	0	0	0	0	10	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1

APPENDIX TABLE 3.—Counts of selected categories of fish larvae, tabulated by station, EASTROPAC I.—*Continued.*

STATION NUMBER	Bathylagus nigrigenys	Leuroglossus stilbius urotranus	Nansenia spp.	Argyrophos sp.	Cyclothone spp.	Diplophos sp.	Ichthyococcus spp	Maurolicus muelleri	Vinciguerria spp.	Bathophilus filifer	Evermannellidae	Macroparalepis macrurus	Sudis atrox	Scopelo saurus spp.	Oxyporhamphus micropterus	Trachypteridae	Auxis spp.	Katsuwonus pelamis	Coryphaena spp.	Naucrates ductor	Howella pammelas	Tetragonurus sp.	Ceratioidei
11.163	0	0	0	0	8	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
.167	0	0	0	0	25	0	0	0	4	0	0	0	1	0	0	0	0	0	0	0	0	0	0
.169	0	0	0	0	3	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0
.171	0	0	0	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.173	0	0	0	0	9	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
.175	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.177	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
.179	0	0	0	0	4	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
.181	0	0	0	0	13	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
.183	0	0	0	0	3	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.185	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.187	0	0	0	0	19	0	0	0	3	0	0	0	0	0	1	0	0	0	0	0	0	0	0
.189	0	0	0	0	20	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.191	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.195	0	0	0	4	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.197	0	0	0	8	32	0	0	0	5	0	2	0	1	0	0	0	1	1	0	0	3	0	0
.199	0	0	0	5	1	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
11.201	0	0	0	0	2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
.203	0	0	0	8	12	0	0	0	3	0	2	1	0	0	0	0	0	0	0	0	0	0	0
.205	0	0	0	22	20	0	2	0	9	3	0	0	0	0	0	0	0	0	0	0	0	0	0
.207	0	0	0	10	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
.209	0	0	0	3	3	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.211	0	0	0	35	4	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0
.213	0	0	0	13	10	1	0	0	12	0	0	0	0	0	0	0	1	3	0	0	1	0	0
.215	0	0	0	3	2	0	0	0	0	0	1	0	0	0	0	0	0	3	0	0	1	0	0
.217	0	0	0	0	3	0	0	0	5	0	0	0	0	0	0	1	0	0	0	0	0	0	1
.219	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.221	0	0	0	0	18	2	0	0	36	0	0	0	0	0	0	0	1	0	1	0	0	0	0
.223	0	0	0	0	8	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.226	0	0	0	0	2	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.228	0	0	0	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0
.234	2	0	0	0	7	2	0	0	8	0	0	0	0	0	0	1	0	0	2	0	1	1	1
.238	0	0	0	0	3	0	0	0	5	0	0	0	0	0	0	0	0	0	1	0	0	0	0
.242	4	0	0	0	13	0	1	0	36	0	0	0	0	0	0	0	0	0	0	0	4	0	0
.246	2	0	0	0	15	0	0	0	36	0	0	0	0	0	0	0	0	0	1	0	1	0	1
.250	1	0	1	0	12	0	0	0	8	0	0	0	0	0	0	1	0	0	1	0	0	0	1
.254	0	0	0	0	5	0	0	0	15	1	0	1	0	0	1	0	0	0	0	0	2	0	0
.258	3	0	1	0	8	0	0	0	46	0	0	0	0	0	0	0	0	0	0	0	1	0	0
.262	2	0	0	0	10	0	0	0	58	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.266	3	0	0	0	3	0	0	0	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.270	4	0	0	0	1	0	0	0	17	0	0	0	0	0	0	0	7	0	0	0	1	0	0
.278	13	0	1	0	13	0	0	0	142	0	0	0	0	0	0	0	0	0	1	0	0	0	0
.282	1	0	0	0	6	0	1	0	20	0	1	0	0	0	0	0	0	0	1	0	0	0	0
.285	9	0	0	0	5	0	0	0	5	0	2	1	0	0	1	0	0	0	1	0	1	0	1
.287	1	0	0	0	4	0	0	0	14	0	1	2	0	0	0	0	0	0	0	0	2	0	2
.289	0	0	0	0	7	4	0	0	5	0	0	0	0	0	0	0	0	0	1	0	18	0	6
.291	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	4	0	0
.293	7	0	1	0	1	0	0	0	2	0	0	0	0	0	0	0	6	0	0	0	14	0	1
.295	10	0	0	0	5	0	0	0	10	0	0	0	0	0	0	1	0	1	1	0	3	0	2
.297	5	0	0	0	3	0	0	0	9	0	0	0	0	0	0	0	2	1	0	0	1	0	4
.299	27	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	5	0	1	0	1	0	1
11.301	1	0	0	0	0	0	0	0	13	0	0	0	0	0	0	0	1	0	1	0	0	0	3
.303	12	0	0	0	2	0	0	0	45	0	0	0	0	0	0	1	0	0	0	0	0	0	1
.306	4	0	0	0	0	0	0	0	64	0	0	0	0	0	0	0	1	0	0	0	0	0	1

APPENDIX TABLE 3.—Counts of selected categories of fish larvae, tabulated by station, EASTROPAC I.—*Continued.*

STATION NUMBER	Bathylagus nigrigenys	Leuroglossus stilbius urotranus	Nansenia spp.	Araiophos sp.	Cyclothone spp.	Diplophos sp.	Ichthyococcus spp.	Maurolicus muelleri	Vinciguerria spp.	Bathophilus filifer	Evermannellidae	Macroparalepis macrurus	Sudis atrox	Scopelo saurus spp.	Oxyporhamphus micropterus	Trachypteridae	Auxis spp.	Katsuwonis pelamis	Coryphaena spp.	Naucrates ductor	Howella pammelas	Tetragonurus sp.	Ceratioidei
11.308	1	0	0	0	2	0	0	0	4	0	0	0	0	0	0	0	0	0	2	0	0	0	1
.310	4	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	1
.312	0	0	0	0	2	1	0	0	59	0	0	1	0	0	0	1	0	0	0	0	0	0	1
.314	5	0	0	0	0	0	0	0	32	1	0	0	0	0	0	0	0	0	1	0	0	0	0
.316	0	0	0	0	0	0	0	0	3	1	0	0	0	0	0	1	0	0	0	0	0	0	0
.318	0	0	0	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.320	1	0	0	0	0	0	0	0	10	0	0	0	0	0	0	1	0	0	0	0	0	0	0
.322	0	0	0	0	2	0	0	0	33	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.324	0	0	0	0	2	0	0	0	19	0	0	1	0	0	0	0	0	0	0	0	0	0	0
.326	0	0	0	0	0	0	0	0	36	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.328	0	0	0	0	9	0	0	0	31	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12.002	3	0	0	0	1	1	0	0	11	1	0	0	0	0	0	0	0	0	0	0	0	0	0
.004	3	0	0	0	0	1	0	0	11	3	0	0	0	0	0	0	0	0	0	0	0	0	0
.006	2	0	0	0	0	3	0	0	12	5	0	0	0	0	0	0	0	0	2	0	0	0	0
.008	3	0	0	0	0	6	0	0	59	2	0	0	0	0	0	0	0	0	1	0	0	0	0
.010	8	0	0	0	0	1	0	0	97	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.012	4	0	0	0	0	8	0	0	16	1	0	0	0	0	0	0	0	0	1	0	0	0	0
.014	4	0	0	0	0	5	0	0	33	2	0	0	0	0	0	0	0	0	0	0	0	0	0
.016	8	0	0	0	0	4	0	0	178	4	0	0	0	0	0	1	0	0	0	0	0	0	2
.018	0	0	0	0	0	0	0	0	199	7	0	0	0	0	1	0	0	0	0	0	0	0	0
.020	3	0	0	0	0	3	0	0	20	4	0	0	0	0	0	0	0	0	1	0	0	0	0
.022	0	0	0	0	0	4	0	0	11	1	0	0	0	0	1	0	0	0	0	0	0	0	0
.024	3	0	0	0	0	8	0	0	234	32	0	0	0	0	0	0	0	0	0	1	0	0	0
.026	4	0	0	0	0	2	0	0	99	3	0	0	0	0	0	0	0	0	0	0	0	0	0
.028	0	0	0	0	0	1	0	0	31	0	0	0	0	0	2	0	3	0	0	0	0	0	1
.030	6	0	0	0	0	0	0	0	12	0	0	0	0	0	0	0	1	0	0	0	0	0	3
.032	0	0	0	0	0	0	0	0	13	1	0	0	0	0	0	0	7	0	0	0	0	0	3
.033	3	0	0	0	0	0	0	0	36	3	0	0	0	0	0	0	6	0	1	0	0	0	0
.035	1	0	0	0	0	1	0	0	72	4	0	0	0	0	5	0	2	0	0	0	0	0	2
.037	9	0	0	0	0	1	0	0	10	1	0	0	0	0	0	0	2	0	0	0	0	0	0
.039	4	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.041	3	0	0	0	0	2	0	0	20	3	0	0	0	0	3	0	1	0	0	0	0	0	0
.043	0	0	0	0	0	0	0	0	28	2	0	0	0	0	3	1	0	0	0	0	0	0	0
.045	0	0	0	0	0	0	0	0	7	0	0	0	0	0	3	0	0	0	0	0	0	0	0
.047	1	0	0	0	0	2	0	0	2	0	0	0	0	0	3	0	1	0	0	0	0	0	1
.049	2	0	0	0	0	2	0	0	52	1	0	0	0	0	0	0	2	0	0	0	0	0	0
.051	0	0	0	0	0	0	0	0	68	0	0	0	0	0	0	0	0	0	0	0	0	0	1
.053	1	0	0	0	0	0	0	0	18	0	0	0	0	0	0	0	2	0	1	0	0	0	0
.055	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	2	0	0	0	0	0	0	0
.057	2	0	0	0	0	0	0	0	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.059	21	0	0	0	12	0	0	0	66	0	0	0	0	0	0	1	0	0	0	0	0	0	0
.061	8	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.063	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.065	11	0	0	0	3	0	0	0	44	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.067	2	0	0	0	4	0	0	0	31	0	0	0	0	0	2	0	19	0	7	0	3	0	1
.069	5	0	0	0	6	0	1	0	11	0	0	0	0	0	0	1	2	0	1	0	7	0	0
.071	9	0	3	0	2	0	0	0	16	0	0	0	0	0	0	0	0	0	1	0	0	0	1
.075	19	0	2	0	6	0	1	0	119	1	2	1	0	0	2	0	0	0	1	0	1	0	2
.077	13	0	1	0	3	0	0	0	23	0	0	0	0	0	2	1	1	0	0	0	4	0	0
.079	17	0	1	0	24	0	0	2	21	0	0	0	0	0	2	0	42	0	0	0	3	0	2
.081	29	0	0	0	15	0	0	0	59	0	0	0	0	0	2	0	30	0	0	0	2	0	1
.084	14	0	0	0	5	0	0	0	6	0	0	0	0	0	0	0	8	0	0	0	0	0	0
.087	13	0	1	0	6	0	0	0	10	0	0	0	0	0	0	0	1	0	0	0	0	0	0

APPENDIX TABLE 3.—Counts of selected categories of fish larvae, tabulated by station, EASTROPAC I.—*Continued.*

STATION NUMBER	Bathylagus nigrigenys	Leuroglossus stilbius urotranus	Nansenia spp.	Araiophos sp.	Cyclothone spp.	Diplophos sp.	Ichthyococcus spp.	Maurolicus muelleri	Vinciguerria spp.	Bathophilus filifer	Evermanellidae	Macroparalepis macrurus	Sudis atrox	Scopelosaurus spp.	Oxyporhamphus micropterus	Trachypteridae	Auxis spp.	Katsuwonis pelamis	Coryphaena spp.	Naucrates ductor	Howella pammelas	Tetragonurus sp.	Cerationdei
12.090	4	0	2	0	2	0	1	7	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.092	0	0	0	0	5	0	0	4	41	0	0	0	0	0	0	0	0	0	0	0	0	0	1
.094	3	0	0	0	8	0	0	1	124	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.097	3	0	0	0	7	0	0	8	46	0	0	0	0	0	0	1	0	0	0	0	0	0	1
12.100	12	0	3	0	4	0	0	0	10	0	0	0	0	0	0	1	1	0	0	0	1	0	0
.103	0	0	0	0	3	0	0	1	33	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.106	26	0	5	0	6	0	0	0	35	0	0	0	0	0	1	1	3	0	0	0	0	0	0
.109	1	0	2	0	7	0	0	0	16	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.112	6	0	1	0	1	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.115	17	0	4	0	0	0	0	0	12	0	0	0	0	0	0	0	36	0	3	0	0	0	1
.118	0	0	0	0	18	0	0	0	141	0	1	0	0	0	1	0	11	0	2	0	3	0	1
.120	0	0	0	0	2	0	0	0	4	0	0	0	0	0	0	0	3	0	0	0	0	0	0
.122	0	0	0	0	1	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.124	0	0	0	0	3	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	1
.126	0	0	0	0	4	0	0	0	18	0	0	0	0	0	0	1	0	0	0	0	0	0	0
.128	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.130	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.132	0	0	0	0	1	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.134	0	0	0	0	10	0	0	0	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.136	0	0	0	0	3	0	0	0	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.138	0	0	0	0	0	0	0	0	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.140	0	0	0	0	3	0	0	0	18	0	0	0	0	0	0	0	0	2	0	0	0	0	2
.142	0	0	0	0	22	2	0	0	86	0	0	0	0	0	0	0	1	1	0	0	0	0	0
.144	0	0	0	0	15	0	0	0	147	0	0	1	0	0	0	0	0	0	0	0	0	0	1
.146	0	0	0	0	0	1	0	0	12	0	0	0	0	0	0	0	0	0	0	0	0	0	1
.148	0	0	0	0	0	3	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.150	0	0	0	0	28	2	0	0	6	1	0	0	0	0	0	0	0	16	1	0	0	0	0
.152	0	0	0	0	9	1	0	0	5	0	2	0	0	0	0	0	0	0	0	0	0	0	0
.154	0	0	0	0	8	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.156	0	0	0	0	7	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	3	0	0
.158	0	0	0	0	42	0	0	0	8	0	0	2	1	1	0	0	0	0	0	0	0	0	0
.160	0	0	0	0	19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.162	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
.164	0	0	0	0	24	1	0	0	15	0	0	0	0	0	0	1	0	0	0	0	0	0	0
.184	0	0	0	0	31	0	0	0	17	0	0	0	0	0	0	0	0	0	0	0	0	0	1
.186	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0
.188	0	0	0	0	2	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.190	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0
.192	0	0	0	0	14	1	1	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.194	0	0	0	0	4	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.196	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
.198	0	0	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12.200	0	0	0	0	3	0	0	0	3	0	1	0	0	0	1	0	13	0	0	0	0	0	0
.203	1	0	0	0	1	0	0	0	3	0	0	0	0	0	0	0	1	0	0	0	0	0	0
.206	4	0	1	0	0	0	0	0	15	1	0	0	0	0	0	0	1	0	0	0	0	0	0
.209	4	0	5	0	3	0	2	0	1	0	0	0	0	0	0	0	2	0	0	0	0	0	0
.212	16	0	0	0	17	0	0	0	17	0	0	0	0	0	0	0	5	0	1	0	0	0	0
.215	7	0	2	0	16	0	2	0	27	0	0	0	0	1	0	2	12	0	1	0	0	0	1
.218	1	0	4	0	9	0	0	2	11	0	0	0	0	0	0	0	6	0	0	0	0	0	4
.221	2	0	3	0	15	0	4	9	16	0	0	0	0	0	0	0	0	0	1	0	0	0	0
.224	0	0	0	0	48	0	0	2	201	0	0	0	0	0	0	0	2	0	0	0	0	0	2
.227	1	0	0	0	8	0	0	5	81	1	0	0	0	1	1	0	1	0	0	0	0	0	1
.230	3	0	6	0	1	0	0	0	4	0	0	0	0	0	0	1	0	0	0	0	0	0	0
.233	45	0	3	0	3	0	1	2	46	0	0	0	0	0	0	0	0	0	0	0	0	0	0

APPENDIX TABLE 3.—Counts of selected categories of fish larvae, tabulated by station, EASTROPAC I.—*Continued.*

STATION NUMBER	*Bathylagus nigrigenys*	*Leuroglossus stilbius urotranus*	*Nansenia* spp.	*Araiophos* sp.	*Cyclothone* spp.	*Diplophos* sp.	*Ichthyococcus* spp.	*Maurolicus muelleri*	*Vinciguerria* spp.	*Bathophilus filifer*	Evermannellidae	*Macroparalepis macrurus*	*Sudis atrox*	*Scopelo saurus* spp.	*Oxyporhamphus micropterus*	Trachipteridae	*Auxis* spp.	*Katsuwonis pelamis*	*Coryphaena* spp.	*Nauerates ductor*	*Howella pammelas*	*Tetragonurus* sp.	Ceratioidei
12.235	25	0	1	0	9	0	1	0	49	0	0	0	0	0	0	0	11	0	0	0	0	0	1
.238	17	0	0	0	7	0	1	0	31	3	0	1	0	0	0	0	17	0	0	0	0	0	0
.240	11	0	1	0	3	2	0	0	14	3	0	1	0	0	0	0	23	0	1	0	2	0	1
.242	1	0	2	0	1	0	0	0	0	7	0	0	0	0	2	0	12	0	1	0	3	0	0
.244	3	0	0	0	8	2	0	0	4	2	0	0	0	0	1	0	9	0	2	0	22	0	5
.246	11	0	0	0	1	1	1	0	0	1	0	0	0	0	0	0	8	0	1	0	7	0	2
.248	1	0	0	0	0	0	0	0	6	0	0	0	0	0	0	1	1	0	2	0	1	0	0
.250	1	0	0	0	1	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.252	1	0	0	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.254	1	0	0	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.256	3	0	0	0	0	1	0	0	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0
.258	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.260	2	0	0	0	4	1	0	0	15	0	0	0	0	0	0	0	0	0	0	0	2	0	2
.262	2	0	0	0	0	5	0	0	97	3	0	0	0	0	0	0	0	0	0	1	0	0	0
.264	2	0	0	0	0	6	0	0	14	1	0	0	0	0	0	0	0	0	1	0	0	0	0
.265	3	0	0	0	0	7	0	0	11	1	0	0	0	0	0	0	0	0	0	0	0	0	0
.268	0	0	0	0	0	2	0	0	103	1	0	0	0	0	0	0	0	0	2	0	0	0	2
.270	1	0	0	0	0	0	0	0	29	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.272	4	0	0	0	0	5	0	0	38	0	0	0	0	0	0	0	0	0	0	0	0	0	2
.274	0	0	0	0	0	4	0	0	36	1	0	0	0	0	0	0	0	0	0	0	0	0	0
.276	0	0	0	0	0	0	0	0	138	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.278	1	0	0	0	0	1	0	0	164	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.280	0	0	0	0	2	0	0	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.282	1	0	0	0	0	2	0	0	21	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.284	0	0	0	0	1	1	0	0	113	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13.001	8	0	0	0	3	0	0	0	90	0	0	0	0	0	0	0	1	0	0	0	0	0	0
.003	14	0	0	0	35	0	0	0	1130	1	1	2	0	0	0	0	0	0	0	0	1	0	0
.005	54	0	0	0	6	1	2	0	300	0	0	0	0	0	0	0	3	0	1	0	0	0	0
.007	9	0	0	0	2	0	0	0	11	0	0	0	0	0	0	0	7	0	0	0	0	0	1
.009	8	0	0	0	1	0	0	0	9	0	0	0	0	0	0	0	7	0	0	0	0	0	0
.011	6	1	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0
.013	18	0	0	0	0	0	1	0	8	0	0	0	0	0	1	0	1	0	0	0	0	0	0
.015	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0
.017	16	0	0	0	1	0	1	0	61	1	0	0	0	0	0	1	8	0	1	0	0	0	0
.019	9	0	0	0	0	0	0	0	82	0	0	0	0	0	9	0	33	0	1	0	0	0	1
.021	4	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	20	0	0	0	0	0	0
.022	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
.028	4	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	1	0	0	0	0	0	0
.030	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	2
.032	0	0	0	0	0	0	0	0	24	0	0	0	0	0	0	0	52	0	0	0	0	0	8
.034	23	0	0	0	1	0	0	0	60	0	0	0	0	0	4	0	41	0	0	0	1	0	3
.036	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.038	28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
.040	20	0	0	0	5	0	2	0	16	1	0	0	0	0	0	0	20	0	0	0	0	0	1
.042	11	0	0	0	5	0	3	0	6	0	0	0	0	0	0	1	4	0	0	0	0	0	0
.044	10	0	0	0	3	0	0	0	11	0	0	0	0	0	0	1	1	0	0	0	0	0	0
.046	10	0	0	0	5	0	0	0	73	4	0	0	0	0	0	1	0	0	1	0	0	0	0
.048	29	0	0	0	19	0	0	0	291	2	0	0	0	0	1	0	1	0	1	0	6	0	4
.050	10	0	0	0	5	0	0	0	36	1	0	0	0	0	0	0	0	0	0	0	0	0	0
.052	13	0	0	0	16	0	1	1	181	0	0	4	0	0	0	0	0	0	1	0	5	0	0
.054	14	0	1	0	9	1	0	5	76	4	0	0	0	0	3	0	11	0	0	0	0	0	1
.056	33	0	1	0	29	0	2	27	454	16	1	1	0	0	0	0	0	0	0	0	6	0	0
.058	6	0	0	0	8	0	1	10	319	1	0	0	0	0	0	0	7	0	0	0	0	0	0

APPENDIX TABLE 3.—Counts of selected categories of fish larvae, tabulated by station, EASTROPAC I.—*Continued.*

STATION NUMBER	Bathylagus nigrigenys	Leuroglossus stilbius urotranus	Nansenia spp.	Araiophos sp.	Cyclothone spp.	Diplophos sp.	Ichthyococcus spp.	Maurolicus muelleri	Vinciguerria spp.	Bathophilus filifer	Evermannellidae	Macropsaralepis macrurus	Sudis atrox	Scopelo saurus spp.	Oxyporhamphus micropterus	Trachypteridae	Auxis spp.	Katsuwonis pelamis	Coryphaena spp.	Naucrates ductor	Howella pammelas	Tetragonurus sp.	Cerataioidei
13.060	5	0	0	0	2	0	2	14	8	0	0	0	0	0	0	0	17	0	0	0	0	0	0
.062	7	0	0	0	0	0	0	3	5	0	0	0	0	0	0	0	1	0	0	0	0	0	0
.064	15	0	0	0	5	0	3	18	45	0	0	0	0	0	0	0	1	0	0	0	0	0	0
.065	2	0	0	0	4	0	1	11	56	0	0	0	0	0	0	0	0	0	0	0	1	0	0
.067	7	0	0	0	2	0	0	0	52	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.069	37	0	1	0	2	0	0	13	45	1	0	0	0	0	0	0	1	0	0	0	0	0	0
.071	37	0	0	0	17	0	0	0	555	6	0	0	0	0	1	0	14	0	4	0	0	0	1
.073	42	0	0	0	13	0	0	0	153	1	0	0	0	0	0	0	3	0	4	0	0	0	5
.075	8	0	0	0	3	0	0	0	18	0	0	0	0	0	0	0	1	0	1	0	0	0	0
.077	0	0	0	0	0	0	0	0	59	2	0	0	0	0	0	0	5	0	0	0	0	0	2
.079	0	0	0	0	1	0	0	0	134	0	0	0	0	0	0	0	5	0	0	0	0	0	1
.081	2	0	0	0	0	0	0	0	164	0	0	1	0	0	0	0	0	0	0	0	0	0	1
.083	5	0	0	0	0	0	0	0	43	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.085	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.087	0	0	0	0	2	0	0	0	64	0	0	0	0	0	0	0	0	0	0	0	0	0	2
.089	0	0	0	0	9	0	0	0	17	0	0	0	0	0	0	0	0	0	0	0	0	0	2
.091	0	0	0	0	0	0	0	0	11	0	0	1	0	0	0	0	2	0	1	0	1	0	0
.093	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.095	6	0	0	0	25	1	0	0	120	2	1	1	0	0	0	0	10	0	0	0	0	0	1
.097	3	0	0	0	11	0	1	0	87	1	1	2	0	0	0	0	1	0	0	0	0	0	0
.099	0	0	0	0	4	1	0	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13.101	3	0	0	0	0	0	1	0	7	0	0	2	0	0	0	0	0	0	1	0	0	0	0
.103	1	0	0	0	36	0	0	0	125	1	2	2	0	0	0	0	0	0	0	0	0	0	6
.105	0	0	0	0	20	0	0	0	30	0	0	1	0	0	0	0	0	0	0	0	0	1	2
.107	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.109	0	0	0	0	1	0	0	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0	1
.111	0	0	0	0	7	0	0	0	11	0	0	0	0	1	0	0	0	0	0	0	0	0	0
.113	0	0	0	0	12	0	0	0	18	0	0	1	1	0	0	1	0	0	0	0	0	0	1
.115	0	0	0	0	4	0	0	0	4	0	1	0	0	0	0	0	0	0	0	0	0	0	0
.117	0	0	0	0	4	1	0	0	4	1	0	0	1	0	0	0	0	0	0	0	0	1	0
.119	0	0	0	0	26	3	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.121	0	0	0	0	10	0	1	0	6	0	0	0	1	0	0	0	0	0	0	0	0	0	0
.123	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.125	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.127	0	0	0	0	13	0	0	0	7	0	0	0	1	0	0	0	0	0	0	0	0	0	0
.129	0	0	0	0	9	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.131	0	0	0	0	4	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.133	0	0	0	0	4	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.135	0	0	0	0	46	0	0	0	10	0	0	0	1	0	0	0	0	0	0	0	0	0	0
.137	0	0	0	0	12	0	0	0	28	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.139	0	0	0	0	3	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.141	0	0	0	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.143	0	0	0	0	21	0	0	0	55	0	0	0	1	0	0	0	5	0	0	0	0	0	0
.145	0	0	0	0	12	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.147	0	0	0	0	1	0	0	0	16	0	0	0	0	0	0	0	0	0	0	0	0	0	3
.149	0	0	0	0	6	0	0	0	8	0	0	0	0	0	0	1	0	0	1	0	0	0	0
.151	0	0	0	0	7	0	0	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.153	0	0	0	0	44	0	0	0	59	0	3	0	0	0	0	0	0	0	0	0	0	0	0
.155	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	2
.157	0	0	0	0	3	0	0	0	5	0	0	0	0	0	0	0	1	0	0	0	0	0	0
.159	0	0	0	0	8	1	0	0	3	0	1	1	0	0	0	0	4	0	0	0	0	0	0
.161	0	0	0	0	11	0	0	0	13	0	0	1	0	0	0	0	0	0	0	0	0	0	0
.163	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.165	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0

APPENDIX TABLE 3.—Counts of selected categories of fish larvae, tabulated by station, EASTROPAC I.—*Continued.*

STATION NUMBER	Bathylagus nigrigenys	Leuroglossus stilbius urotranus	Nansenia spp.	Araiophos sp.	Cyclothone spp.	Diplophos sp.	Ichthyococcus spp.	Maurolicus muelleri	Vinciguerria spp.	Bathophilus filifer	Evermannellidae	Macropsaralepis macrurus	Sudis atrox	Scopelo saurus spp.	Oxyporhamphus micropterus	Trachypteridae	Auxis spp.	Katsuwonis pelamis	Coryphaena spp.	Naucrates ductor	Howella pammelas	Tetragonurus sp.	Ceratioidei
13.167	0	0	0	0	5	0	0	0	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.169	0	0	0	0	7	0	0	0	76	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.171	0	0	0	0	2	0	0	0	16	0	0	0	0	0	0	0	13	0	0	0	0	0	0
.173	7	0	0	0	0	0	0	0	4	0	0	1	0	0	0	0	6	0	0	0	0	0	0
.175	22	0	0	0	6	0	0	0	169	0	1	1	0	0	0	0	45	0	0	0	1	0	1
.179	15	0	0	0	6	0	0	0	105	0	0	0	0	0	1	0	7	0	0	0	0	0	1
.183	50	0	3	0	3	0	0	0	36	0	0	0	0	0	0	0	6	0	0	0	0	0	0
.187	43	0	2	0	3	0	0	0	26	0	0	0	0	0	0	0	0	0	1	0	0	0	0
.191	10	0	6	0	25	0	0	5	118	0	0	0	0	0	1	0	7	0	0	0	0	0	1
.195	10	0	0	0	8	0	0	0	194	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.199	5	0	0	0	1	0	0	2	25	0	0	0	0	0	0	0	0	0	0	0	0	0	1
13.203	3	0	1	0	2	0	0	9	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.207	11	0	0	0	11	0	0	7	143	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.211	5	0	0	0	6	0	2	1	27	0	0	0	0	0	0	0	1	0	0	0	0	0	0
.215	6	0	0	0	3	0	0	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.219	9	0	0	0	1	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.223	2	0	0	0	3	0	0	2	15	0	0	0	0	0	0	0	0	0	0	0	0	0	1
.227	9	0	0	0	14	0	0	4	20	0	0	0	0	0	3	0	1	0	0	0	0	0	0
.231	9	0	1	0	5	0	0	0	29	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.235	9	0	1	0	3	0	2	0	31	0	0	0	0	0	1	0	1	0	0	0	0	0	0
.237	6	0	0	0	12	0	2	0	33	0	0	1	0	0	0	0	7	0	1	1	0	0	0
.239	3	0	0	0	7	0	3	0	34	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.241	2	0	0	0	1	0	0	0	4	0	0	0	0	0	0	0	1	0	0	0	0	0	0
.243	11	0	0	0	0	0	0	0	13	0	0	0	0	0	0	0	0	0	0	0	0	0	1
.245	7	0	0	0	1	0	0	0	13	0	0	0	0	0	0	0	0	0	0	0	0	0	1
.247	9	0	0	0	0	0	0	0	11	0	0	0	0	0	0	1	2	0	1	0	0	0	0
.249	1	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.251	6	0	0	0	0	0	0	0	10	2	0	0	0	0	0	0	3	0	0	0	0	0	0
.253	20	0	0	0	0	0	0	0	22	4	0	0	0	0	0	0	0	0	0	0	0	0	0
.255	7	0	0	0	0	0	0	0	29	0	0	0	0	0	1	0	15	0	1	0	0	0	3
.257	8	0	0	0	0	0	0	0	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0
.259	17	0	0	0	0	0	0	0	18	0	0	0	0	0	0	0	5	0	0	0	0	0	0
.261	8	0	0	0	1	0	0	0	36	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.263	19	0	0	0	0	0	0	0	54	0	0	0	0	0	1	0	11	0	0	0	0	0	0
.265	6	0	0	0	0	1	0	0	12	8	0	0	0	0	0	0	1	0	0	0	0	0	1
.266	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	2	0	0	0	0	0	0
.268	3	0	0	0	0	0	0	0	26	0	0	0	0	0	0	0	1	0	0	0	0	0	0
.270	4	0	0	0	0	2	0	0	18	3	0	0	0	0	12	0	1	0	0	0	0	0	1
.272	2	0	0	0	0	0	0	0	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0
.274	1	0	0	0	0	3	0	0	49	6	0	0	0	0	3	0	1	0	1	0	0	0	0
.276	5	0	0	0	0	2	0	0	89	3	0	0	0	0	3	0	19	0	0	0	0	0	0
.278	1	0	0	0	0	1	0	0	20	2	0	0	0	0	0	0	0	0	0	0	0	0	0
.280	8	0	0	0	0	3	0	0	5	1	0	0	0	0	0	0	0	0	0	0	0	0	0
.282	0	0	0	0	0	3	0	0	33	6	0	0	0	0	1	0	0	0	1	0	0	0	0
.284	18	0	0	0	1	0	0	0	60	1	0	0	0	0	2	0	0	0	0	0	0	0	1
13.318	13	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1
.320	8	0	0	0	0	0	0	0	16	0	0	0	0	0	0	0	13	0	0	0	0	0	0
.322	0	0	0	0	0	0	0	0	5	0	0	0	0	0	1	0	9	0	1	0	0	0	0
.324	10	0	0	0	0	0	0	0	4	0	0	0	0	0	2	0	4	0	0	0	0	0	0
.326	13	0	0	0	1	0	0	0	9	0	0	0	0	0	0	0	18	0	0	0	0	0	1
.328	2	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	7	0	0	0	0	0	0
.330	4	0	0	0	0	0	0	0	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.332	11	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	2	0	0	0	0	0	1

APPENDIX TABLE 3.—Counts of selected categories of fish larvae, tabulated by station, EASTROPAC I.—*Continued.*

STATION NUMBER	Bathylagus nigrigenys	Leuroglossus stilbius urotranus	Nansenia spp.	Araiophos sp.	Cyclothone spp.	Diplophos sp.	Ichthyococcus spp.	Maurolicus muelleri	Vinciguerria spp.	Bathophilus filifer	Evermannellidae	Macropsaralepis macrurus	Sudis atrox	Scopelo saurus spp.	Oxyporhamphus micropterus	Trachypteridae	Auxis spp.	Katsuwonis pelamis	Coryphaena spp.	Naucrates ductor	Howella pammelas	Tetragonurus sp.	Cerataioidei
13.334	37	0	0	0	2	0	0	0	19	1	0	0	0	0	0	1	0	0	0	0	0	0	2
.338	9	0	1	0	1	0	0	0	48	0	0	0	0	0	2	0	5	0	0	0	0	0	0
.340	4	0	0	0	3	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	1	0	0
.342	9	0	1	0	8	0	0	2	14	0	0	0	0	0	0	0	4	0	0	0	0	0	0
14.001	9	30	0	0	3	0	0	0	94	0	0	0	0	0	0	0	8	0	0	0	0	0	0
.006	3	29	0	0	3	0	0	0	15	0	0	0	0	0	0	0	10	0	0	0	0	0	0
.008	9	25	1	0	0	0	0	0	4	0	0	0	0	0	0	0	1	0	1	0	0	0	1
.010	1	13	0	0	3	0	0	0	16	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.012	2	4	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.014	3	39	0	0	0	0	0	0	4	0	0	0	0	0	0	0	2	0	0	0	0	0	0
.016	10	9	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
.017	11	6	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
.018	24	17	0	0	6	0	0	0	42	0	0	0	0	0	0	0	0	0	0	0	0	0	1
.020	6	0	0	0	0	0	0	0	10	0	0	0	0	0	0	1	0	0	0	0	0	0	0
.022	1	6	0	0	3	0	0	0	19	0	0	0	0	0	0	0	0	0	0	0	0	0	1
.024	1	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.027	17	6	0	0	1	0	0	0	30	0	0	0	0	0	0	0	6	0	0	0	0	0	0
.029	13	11	0	0	1	0	0	0	41	0	0	0	0	0	0	0	0	0	2	0	0	0	2
.031	29	1	0	0	2	0	0	0	41	0	0	0	0	1	0	0	1	0	1	0	0	0	0
.033	20	1	0	0	0	0	1	0	4	0	0	0	0	0	0	0	0	0	0	1	0	0	0
.040	48	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	6	0	2	0	0	0	0
.043	59	6	0	0	3	0	0	0	14	1	0	0	0	6	0	0	22	0	0	0	0	0	5
.047	49	62	0	0	0	0	0	1	2	0	0	0	0	0	2	0	9	0	1	1	0	0	0
.051	20	205	0	0	2	0	0	0	25	0	0	0	0	0	0	0	46	0	0	1	0	0	0
.055	2	152	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.060	0	139	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.066	0	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.069	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.076	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.078	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.081	2	0	0	0	3	0	0	0	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.084	0	2	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	1
.086	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.088	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.091	2	0	0	0	8	0	0	0	32	1	0	0	0	0	0	0	0	0	1	0	0	0	2
.095	2	0	0	0	2	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1
.099	2	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14.103	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.106	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.110	0	0	0	0	5	0	0	0	3	0	0	0	0	0	0	0	0	0	0	1	0	0	0
.112	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.114	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.115	2	0	0	0	1	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	2
.117	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
.118	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.120	1	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	1	0	0	0	0	0	0
.122	2	0	0	0	0	0	0	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0	1
.123	7	0	0	0	0	0	0	0	22	0	0	0	0	0	0	0	0	0	0	0	0	0	2
.124	7	0	0	0	18	0	0	0	58	0	0	0	0	0	0	1	0	0	1	0	0	0	8
.126	3	0	0	0	3	0	1	0	16	0	0	0	0	0	0	0	0	0	0	0	0	0	1
.127	5	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.128	5	0	0	0	4	0	0	0	56	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.130	3	0	0	0	2	0	0	0	42	0	0	0	0	0	0	0	4	0	0	0	0	0	0

APPENDIX TABLE 3.—Counts of selected categories of fish larvae, tabulated by station, EASTROPAC I.—*Continued.*

STATION NUMBER	Bathylagus nigricaudus	Leuroglossus stilbius ventralis	Nansenia spp.	Araiophos sp.	Cyclothone spp.	Diplophos sp.	Ichthyococcus spp.	Maurolicus muelleri	Vinciguerria spp.	Bathophilus filifer	Evermannellidae	Macroparalepis macrurus	Sudis atrox	Scopelosaurus spp.	Oxyporhamphus micropterus	Trachypteridae	Auxis spp.	Katsuwonis pelamis	Coryphaena spp.	Naucrates ductor	Howella pammelas	Tetragonurus sp.	Ceratioidei
14.131	0	0	0	0	0	0	0	0	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.132	8	0	0	0	0	0	0	0	17	0	0	0	0	0	0	1	0	0	1	0	0	0	2
.134	0	0	0	0	1	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	1
.136	1	0	0	0	0	0	0	0	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.138	9	0	0	0	2	0	0	0	90	0	0	0	0	0	0	0	9	0	0	0	0	0	0
.142	1	0	0	0	0	0	0	0	53	4	0	0	0	0	0	1	0	0	0	0	0	0	1
.146	16	30	0	0	0	0	0	0	111	1	0	0	0	0	0	0	8	0	0	0	0	0	0
.150	33	1	0	0	2	0	0	1	45	0	0	0	0	0	4	1	5	0	0	0	0	0	0
.154	28	6	0	0	9	0	1	0	372	0	0	0	0	0	1	0	7	0	0	0	0	0	3
.158	0	0	0	0	4	0	0	1	219	1	0	0	0	0	0	0	0	0	0	0	0	0	1
.164	8	0	0	0	4	0	1	0	48	0	0	0	0	3	0	0	6	0	0	0	0	0	1
.172	1	0	0	0	2	0	0	5	90	0	0	0	0	0	0	0	12	0	0	0	0	0	0
.174	3	0	0	0	0	0	1	3	33	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.177	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.183	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.188	34	0	0	0	4	0	0	9	155	1	0	0	0	0	2	0	36	0	1	0	0	0	1
.194	161	0	0	0	7	0	0	22	98	0	0	0	0	0	0	1	22	0	1	0	0	0	4
.195	54	0	0	0	0	0	0	0	16	0	0	0	0	0	0	0	0	0	2	0	0	0	0
.199	15	0	0	0	5	0	0	0	196	0	0	0	0	0	0	1	20	0	0	0	0	0	3
14.203	3	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	1	0	0	0	0	1
.209	5	0	0	0	0	0	0	0	2	0	0	0	0	0	10	0	0	0	0	0	0	0	0
.213	34	0	0	0	10	0	0	0	86	0	0	0	0	0	0	0	11	0	1	0	0	0	0
.218	27	0	0	0	1	0	0	0	38	0	0	0	0	0	2	1	4	0	2	0	0	0	0
.220	2	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	13	0	0	0	0	0	0
.222	2	0	0	0	0	0	0	0	4	2	0	0	0	0	0	0	1	0	0	0	0	0	0
.224	9	3	0	0	6	0	0	0	31	1	0	0	0	0	0	0	7	0	0	0	1	0	0
.228	2	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	2	0	0	0	0	0	0
.230	1	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	9	0	0	0	0	0	0
.232	9	0	0	0	0	0	0	0	48	0	0	0	0	0	0	0	12	0	0	0	0	0	0
.234	9	0	0	0	2	0	0	0	867	0	0	0	0	0	0	1	161	1	0	0	0	0	0
.236	3	0	0	0	0	0	0	0	16	0	0	0	0	0	0	1	10	0	0	0	0	0	0
.240	21	0	0	0	0	1	0	4	31	0	0	0	0	0	0	0	85	0	0	0	0	0	1
.243	24	0	0	0	1	0	0	7	19	0	0	0	0	0	0	0	5	0	0	0	0	0	0
.247	4	0	0	0	0	0	0	13	39	0	0	0	0	0	0	0	1	0	0	0	0	0	0
.251	8	3	0	0	1	0	0	1	23	0	0	0	0	0	0	0	1	0	0	0	0	0	6
.255	1	209	0	0	10	0	0	0	76	0	0	0	0	0	0	0	59	0	1	0	0	0	6
.259	16	0	0	0	0	0	0	0	40	1	0	0	0	0	0	0	0	0	0	0	0	0	5
.263	2	0	0	0	2	0	0	0	231	0	0	0	6	0	0	0	0	0	0	0	0	0	2
.267	3	0	0	0	0	0	0	0	33	0	0	0	0	0	0	1	0	0	2	0	0	0	3
.276	9	0	0	0	6	0	0	0	52	0	0	0	0	0	0	0	0	0	2	0	0	0	1
.280	7	0	0	0	3	0	0	0	59	2	0	0	0	0	0	0	0	0	2	0	0	0	6
.283	3	0	0	0	0	0	0	0	13	0	0	0	0	0	0	0	0	0	1	0	0	0	1
.287	5	0	0	0	3	0	0	0	59	0	0	0	0	0	0	0	0	0	0	0	0	0	9
.291	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.295	4	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	63	0	2	0	0	0	5
.300	1	286	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
.303	3	490	0	0	2	0	0	0	8	0	0	0	0	0	0	0	0	0	1	0	1	0	1
.306	12	22	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.310	70	21	0	0	0	0	0	9	12	1	0	0	0	0	0	0	0	0	0	0	0	0	0
.314	24	0	0	0	1	0	0	0	24	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.318	32	0	0	0	2	0	0	2	66	0	0	0	0	0	0	1	0	0	0	0	0	0	1
.323	11	0	0	0	0	0	0	0	24	1	0	0	0	0	0	0	0	0	0	0	0	0	0
.326	15	12	0	0	1	0	0	0	65	0	0	0	0	0	0	0	8	0	0	0	0	0	0
.330	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0

APPENDIX TABLE 4.—Summary of occurrences and numbers of larvae of eight families, limited in distribution to a broad coastal band or around offshore islands.

STATION NUMBER	Clupeidae	Engraulidae	Synodontidae	Carangidae	Serranidae	Labridae	Gobiidae	Scorpaenidae
11.076	0	0	0	1	0	0	0	0
11.246	0	0	0	0	0	0	0	1
12.020	0	0	0	0	0	1	0	0
.024	0	0	0	1	4	0	0	0
.026	0	0	0	0	10	0	0	0
.028	0	0	0	0	0	0	3	3
.030	0	0	0	0	0	0	1	1
.031	0	0	0	0	0	0	7	0
.033	1	2	1	2	1	2	1	1
.035	0	0	0	5	0	0	3	1
.041	0	0	0	1	0	0	1	0
.059	0	0	0	0	0	0	1	0
12.221	0	0	0	0	0	0	0	2
.256	0	0	0	0	0	1	0	0
.262	0	0	0	1	0	0	0	0
.264	0	0	0	1	0	0	0	0
.268	0	0	0	1	0	0	0	0
13.003	0	0	0	1	0	0	1	0
.005	0	0	0	0	0	0	1	1
.007	0	0	0	0	0	0	0	1
.011	0	0	0	0	0	0	1	0
.019	0	13	1	70	49	2	47	12
.021	2	7	0	11	3	0	3	1
.030	0	0	0	0	0	0	1	2
.032	0	0	0	0	0	0	12	8
.034	0	0	0	0	0	0	23	3
.040	0	0	0	0	0	0	0	1
.042	0	0	0	0	0	1	0	0
.054	0	0	0	0	1	0	0	0
.056	0	0	0	0	0	0	1	0
.062	0	0	0	0	0	2	0	0
13.235	0	0	0	0	0	0	0	1
.237	0	0	0	1	0	0	0	0
.239	0	0	0	0	0	0	2	0
.245	0	0	0	0	0	0	13	0
.247	0	0	0	0	0	0	5	0
.249	0	0	0	0	0	0	1	0
.253	0	0	0	0	0	0	72	0
.255	2	0	1	2	0	0	2	3
.257	0	0	0	0	0	0	0	1
.261	0	0	0	0	0	0	41	0
.263	0	2	0	0	0	0	0	2
.265	0	0	0	0	0	0	0	1
.266	0	0	0	0	0	1	0	0
.268	0	0	0	0	0	1	0	0
.274	0	0	0	0	0	0	3	0
.276	0	0	0	0	0	0	2	0
13.320	1	0	0	0	0	1	7	3
.328	0	0	0	0	0	0	2	1
.330	0	0	0	0	0	0	4	0
.334	0	0	0	0	0	0	1	0
.338	0	0	0	0	0	0	17	0
14.001	0	5	1	1	84	0	55	11
.006	0	0	0	0	1	0	5	1
.008	0	0	0	0	0	0	2	5
.010	0	0	0	0	0	0	3	2
.012	0	0	1	0	0	1	1	0
.014	6	0	0	4	0	0	11	1
.016	0	0	0	34	0	0	0	0
.017	0	0	0	0	0	1	0	0
.018	0	0	0	0	0	3	10	4
.020	0	0	0	0	0	1	1	1
.022	0	0	0	0	14	0	2	1
.024	0	4	0	6	5	0	0	6
.027	0	0	3	5	1	4	36	9
.029	0	3	7	2	12	0	9	37
.031	0	0	3	0	3	1	18	4
.033	0	0	0	1	0	1	1	2
.040	0	0	0	6	5	0	1	0
.043	0	0	0	0	0	1	2	0
.047	0	0	0	1	0	1	0	3
.051	0	0	0	1	0	0	0	0
.055	0	0	0	0	0	1	0	0
.060	0	52	0	2	0	0	0	0
.066	0	11	0	0	0	0	0	0
.069	0	97	0	0	0	0	0	0
.076	0	11	0	0	0	0	0	0
14.106	0	3	0	0	0	0	0	0
.110	0	0	0	1	0	0	0	0
.154	0	0	0	0	5	0	0	1
.158	5	0	0	1	8	0	3	4
.164	0	8	5	0	0	8	0	1
.172	2	0	0	11	22	3	0	1
.174	1	0	0	0	4	2	1	1
.177	60	3	0	2	6	1	1	0
.194	1	0	0	1	0	1	0	0
.195	0	0	0	0	2	0	0	0
.199	0	0	0	2	0	0	1	0
14.209	0	0	0	0	0	1	0	0
.213	0	0	0	0	0	0	3	0
.220	0	0	0	0	0	1	1	0
.222	0	0	0	0	0	1	0	0
.224	0	0	0	3	0	0	0	1
.228	0	0	0	0	1	0	0	4
.230	0	0	0	2	0	0	0	2
.232	0	0	0	0	0	0	2	0
.234	0	0	0	0	3	0	8	3
.236	0	0	0	0	0	0	1	0
.240	0	0	0	0	0	0	1	1
.243	0	0	0	0	0	0	1	0
.247	0	0	0	0	0	0	2	0
14.303	0	0	0	0	1	1	0	0
.314	0	0	0	0	0	0	0	1
.318	0	0	10	0	4	2	43	5
.323	0	0	0	0	1	0	3	1
.326	0	0	13	0	2	0	23	0

APPENDIX TABLE 5.—Numbers and kinds of larvae of Gempylidae-Trichiuridae obtained in EASTROPAC I collections.

STATION NUMBER	Nealotus tripes	Gempylus serpens	Diplospinus multistriatus	Lepidopus sp.	Other	STATION NUMBER	Nealotus tripes	Gempylus serpens	Diplospinus multistriatus	Lepidopus sp.	Other
11.056	0	1	0	0	0	13.107	0	0	1	0	0
.064	0	0	1	0	0	.119	1	3	0	0	0
.072	0	1	0	0	0	.137	0	2	0	0	0
11.114	1	0	0	0	0	.139	0	0	1	0	0
.138	1	0	0	0	0	.147	0	0	1	0	0
.140	1	0	0	0	0	.153	0	0	1	0	0
.146	0	1	0	0	0	.159	0	2	0	0	0
.158	1	0	0	0	0	.167	1	0	0	0	0
.159	1	0	0	0	0	.171	0	1	0	1	0
11.213	0	1	0	0	0	.173	1	0	0	0	0
.219	0	1	0	0	0	.175	1	0	0	0	0
.228	1	0	0	0	0	.179	0	3	0	0	0
.234	0	1	0	0	0	.187	0	0	0	0	2
.295	0	2	0	0	0	.191	0	1	0	0	0
.297	0	2	0	0	0	13.235	1	0	0	0	0
11.318	0	0	5	0	0	.245	0	1	0	0	0
.320	0	0	1	0	0	.280	0	0	0	0	1
.324	0	0	1	0	0						
.326	0	0	2	0	0	14.001	0	1	0	0	0
						.010	1	0	0	0	0
12.004	0	1	0	0	0	.012	0	0	0	1	0
.014	0	1	0	0	0	.029	1	0	1	1	0
.020	0	1	0	0	0	.031	1	0	0	0	0
.047	0	2	0	0	0	.095	0	0	1	0	0
.081	0	1	0	0	0	14.122	0	0	1	0	0
12.115	0	0	0	0	1	.123	0	0	1	0	0
.118	6	0	0	0	1	.124	1	1	1	0	0
.120	1	0	0	0	0	.126	1	0	1	0	0
.144	0	1	0	0	0	.127	0	0	3	0	0
.150	0	3	0	0	0	.128	0	0	6	0	0
.152	0	1	0	0	0	.130	0	0	3	0	0
.158	0	1	0	0	0	.131	0	0	1	0	0
.188	0	1	0	0	0	.134	1	1	0	0	0
12.246	0	2	0	0	0	.138	3	0	0	0	0
.260	0	1	0	0	0	.142	2	0	0	0	0
.262	0	1	0	0	0	.146	2	0	0	0	0
.272	0	1	0	0	0	.150	1	0	0	0	0
.276	0	1	0	0	0	.164	1	0	0	0	0
						.188	1	0	0	0	0
13.048	1	2	0	0	0	.194	0	0	0	2	0
.054	0	0	0	17	0	.195	1	0	0	0	0
.056	2	0	0	0	0	14.222	1	0	0	0	0
.071	6	0	0	0	0	.224	1	0	0	0	0
.073	7	0	0	0	0	.234	8	0	0	1	0
.075	2	0	0	0	0	.240	1	0	0	0	0
.077	3	0	0	0	0	.259	3	0	0	0	0
.081	8	0	0	0	0	.280	1	4	1	0	0
.083	0	1	0	0	0	.283	0	0	1	0	0
.095	0	0	7	0	0	.287	1	2	0	0	0
.097	0	1	4	0	0	.295	0	1	0	0	0
.101	0	1	6	0	0	14.318	1	0	0	2	0
.103	0	0	7	0	0	.326	0	0	0	1	0
.105	0	0	2	0	0	.330	1	0	0	0	0

APPENDIX TABLE 6.—Numbers and kinds of flatfish (Pleuronectiformes) larvae obtained in EASTROPAC I collections.

Station number	Bothus leopardinus	Citharichthus - Etropus	Cyclopsetta sp.	Engyophrys sancti-laurentii	Syacium ovale	Symphurus spp.	Other Pleuronectiformes	Station number	Bothus leopardinus	Citharichthys - Etropus	Cyclopsetta sp.	Engyophrys sancti-laurentii	Syacium ovale	Symphurus spp.	Other Pleuronectiformes
12.028	0	0	0	0	0	1	0	14.001	1	5	0	1	0	35	0
.030	0	1	0	0	0	0	0	.006	0	1	0	0	0	2	0
.031	0	0	0	0	2	6	0	.008	0	1	0	0	0	3	0
.033	4	1	0	0	6	6	0	.010	0	1	0	1	0	1	0
.035	0	0	0	0	0	3	0	.014	0	2	0	0	0	4	0
.045	0	0	0	0	0	1	0	.016	0	0	0	0	0	5	1
								.017	0	0	0	0	0	2	0
								.018	0	1	0	0	0	1	0
13.007	0	0	0	0	1	0	0	.020	0	0	0	0	0	5	0
.009	0	1	0	0	0	0	0	.022	1	3	0	0	0	1	0
.011	0	1	0	0	0	0	0	.024	0	1	0	0	0	9	0
.013	0	0	0	0	0	5	0	.027	1	6	0	1	3	9	0
.015	1	0	0	0	0	1	0	.029	1	5	0	0	2	24	0
.019	6	1	1	0	25	31	1	.031	0	0	0	1	0	30	0
.021	2	2	0	0	13	8	0	.033	0	0	0	1	0	2	0
.030	0	0	0	0	0	4	0	.040	0	1	0	0	2	4	0
.032	0	0	0	0	0	8	0	.047	0	0	0	0	0	1	0
.034	0	0	0	2	4	9	0	.055	0	1	0	0	0	2	0
.036	0	0	0	0	1	0	0	14.164	1	0	0	0	0	1	0
.040	1	2	0	1	0	3	0	.174	0	0	0	0	0	1	0
.042	1	0	1	0	6	1	0	.183	0	0	0	0	0	1	0
.054	1	0	0	0	0	0	0	.194	0	0	0	0	0	1	0
13.245	1	0	0	0	0	0	0	.195	0	0	0	0	0	1	0
.251	0	0	0	0	0	1	0	.199	0	0	0	0	0	1	0
.253	4	0	0	0	0	4	0	14.209	0	0	0	0	0	1	0
.255	3	1	0	0	0	9	0	.213	0	0	0	0	1	0	0
.257	0	0	0	0	0	1	0	.220	0	0	0	0	0	3	0
.259	0	0	0	0	1	1	0	.228	0	1	0	0	0	3	0
.261	1	0	0	0	1	1	0	.230	0	0	0	0	0	1	0
.263	1	0	0	0	3	8	0	.232	0	0	0	0	1	0	0
.265	0	0	0	0	2	1	0	.234	2	0	2	0	3	1	0
13.318	2	0	0	0	1	1	0	.236	1	0	0	0	0	2	0
.320	2	0	0	0	0	2	0	.240	0	0	0	0	1	0	0
.322	5	0	0	0	0	0	0	.259	2	0	0	0	0	0	0
.324	0	0	0	0	1	0	0	.295	0	1	0	0	0	0	0
.326	0	0	0	0	1	2	0	14.300	0	0	0	0	1	0	0
.328	0	0	0	0	0	1	0	.303	1	1	0	0	0	1	0
.334	1	0	0	0	0	0	0	.306	0	0	0	0	0	1	0
								.314	0	0	0	0	0	1	0
								.318	1	2	0	1	0	19	0
								.323	1	3	0	0	2	2	0
								.326	1	4	0	0	0	3	0
								.330	0	0	0	0	0	1	0

APPENDIX TABLE 7.—Standardized haul factors (SHF): These factors are used to adjust original counts of larvae to the comparable standard of numbers of larvae in 10 m^3 of water strained per meter of depth fished.

Station	SHF	Station	SHF	Station	SHF	Station	SHF	Station	SHF
11.022	3.06	11.156	2.74	11.291	3.46	12.061	3.33	12.192	3.27
11.025	2.87	11.158	3.12	11.293	2.93	12.063	3.27	12.194	3.45
11.027	2.38	11.159	2.64	11.295	3.16	12.065	3.23	12.196	3.32
11.030	2.47	11.161	3.35	11.297	2.86	12.067	3.36	12.198	3.40
11.032	3.01	11.163	2.64	11.299	3.57	12.068	3.39	12.200	3.18
11.034	3.64	11.167	2.97	11.301	3.31	12.071	3.34	12.203	3.29
11.036	3.04	11.169	3.27	11.303	3.19	12.075	3.33	12.206	3.53
11.038	2.80	11.171	2.92	11.306	3.22	12.077	3.42	12.209	3.51
11.040	3.32	11.173	2.94	11.308	3.15	12.079	3.56	12.212	3.32
11.044	2.81	11.175	3.47	11.310	3.19	12.091	3.53	12.215	3.27
11.046	3.24	11.177	1.36	11.312	3.42	12.084	3.73	12.218	3.02
11.048	3.08	11.179	3.37	11.314	3.18	12.087	3.86	12.221	3.07
11.050	2.36	11.181	2.74	11.316	2.84	12.090	3.10	12.224	2.58
11.052	2.86	11.183	2.92	11.318	3.27	12.092	2.55	12.227	2.96
11.054	2.54	11.185	3.19	11.320	3.34	12.094	2.29	12.230	3.72
11.056	2.90	11.187	2.75	11.322	3.01	12.097	3.01	12.233	2.66
11.058	3.28	11.189	3.00	11.324	3.02	12.100	2.48	12.235	3.56
11.060	3.15	11.191	3.79	11.326	2.84	12.103	3.28	12.238	3.21
11.062	3.72	11.195	3.11	11.328	2.62	12.106	3.55	12.240	3.22
11.064	3.01	11.197	3.14			12.109	3.39	12.242	3.41
11.066	2.12	11.199	2.46	12.002	3.12	12.112	3.43	12.244	3.36
11.068	2.62	11.201	3.27	12.004	3.02	12.115	3.48	12.246	3.14
11.070	2.25	11.203	3.09	12.006	3.31	12.118	2.45	12.248	3.07
11.072	3.43	11.205	3.20	12.008	3.08	12.120	3.46	12.250	2.49
11.076	2.92	11.207	3.65	12.010	3.13	12.122	3.43	12.252	2.33
11.080	2.45	11.209	3.06	12.012	3.17	12.124	3.17	12.254	3.30
11.084	2.70	11.211	3.39	12.014	3.28	12.126	3.47	12.256	3.26
11.088	3.19	11.213	2.87	12.016	3.17	12.128	3.30	12.258	3.26
11.094	3.61	11.215	3.13	12.018	3.13	12.130	3.35	12.260	3.51
11.098	1.78	11.217	2.90	12.020	3.12	12.132	3.38	12.262	2.98
11.102	2.72	11.219	3.36	12.022	3.43	12.134	3.29	12.264	3.38
11.106	1.36	11.221	2.92	12.024	3.11	12.136	3.22	12.265	3.27
11.110	2.95	11.223	3.71	12.026	3.30	12.138	3.38	12.268	3.35
11.114	3.35	11.226	3.05	12.028	3.44	12.140	3.00	12.270	3.36
11.118	4.65	11.228	3.29	12.030	3.44	12.142	3.42	12.272	3.12
11.120	3.68	11.234	3.65	12.032	3.32	12.144	3.20	12.274	3.28
11.124	3.67	11.238	3.41	12.033	3.21	12.146	4.36	12.276	3.34
11.128	2.85	11.242	3.77	12.035	3.35	12.148	3.21	12.278	3.00
11.130	3.80	11.246	3.01	12.037	3.20	12.150	3.14	12.280	3.39
11.132	3.37	11.250	2.77	12.039	3.47	12.152	3.17	12.282	3.58
11.134	3.22	11.254	2.51	12.041	3.42	12.154	3.27	12.284	3.41
11.136	3.24	11.258	2.86	12.043	3.33	12.156	3.28		
11.138	3.38	11.262	3.23	12.045	3.35	12.158	3.22	13.001	2.26
11.140	2.77	11.266	2.91	12.047	3.42	12.160	3.49	13.003	2.45
11.142	3.35	11.270	3.69	12.049	3.39	12.162	3.21	13.005	1.42
11.146	3.25	11.278	3.09	12.051	3.31	12.164	2.98	13.007	2.42
11.148	2.54	11.282	3.99	12.053	3.27	12.184	3.22	13.009	2.56
11.150	3.45	11.285	3.20	12.055	2.84	12.186	3.22	13.011	3.68
11.152	2.59	11.287	3.45	12.057	3.22	12.188	3.35	13.013	2.29
11.154	3.40	11.289	3.12	12.059	3.41	12.190	3.39	13.015	2.76

APPENDIX TABLE 7.—Standardized haul factors (SHF): These factors are used to adjust original counts of larvae to the comparable standard of numbers of larve in 10 m^3 of water strained per meter of depth fished.—*Continued.*

Station	SHF	Station	SHF	Station	SHF	Station	SHF	Station	SHF
13.017	2.16	13.119	2.67	13.249	3.46	14.047	4.10	14.203	3.15
13.019	1.88	13.121	3.14	13.251	3.46	14.051	2.93	14.209	3.23
13.021	2.12	13.123	3.06	13.253	3.13	14.055	3.77	14.213	3.26
13.022	2.72	13.125	3.50	13.255	3.58	14.060	3.58	14.218	2.87
13.028	1.53	13.127	3.30	13.257	3.68	14.066	3.81	14.220	3.42
13.030	2.50	13.129	4.01	13.259	3.42	14.069	3.65	14.222	3.64
13.032	3.05	13.131	3.64	13.261	1.85	14.076	3.61	14.224	3.77
13.034	3.21	13.133	3.84	13.263	3.49	14.078	3.64	14.228	3.87
13.036	2.34	13.135	2.51	13.265	3.29	14.081	3.39	14.230	2.96
13.038	2.25	13.137	2.58	13.266	3.31	14.084	3.86	14.232	2.70
13.040	2.85	13.139	3.57	13.268	3.47	14.086	3.95	14.234	0.72
13.042	2.74	13.141	3.36	13.270	3.30	14.088	3.54	14.236	2.96
13.044	2.58	13.143	3.23	13.272	3.06	14.091	3.08	14.240	3.43
13.046	3.08	13.145	3.49	13.274	3.73	14.095	3.87	14.243	3.55
13.048	2.71	13.147	3.58	13.276	3.54	14.099	3.70	14.247	3.52
13.050	3.02	13.149	3.56	13.278	3.16	14.103	3.57	14.251	3.49
13.052	2.91	13.151	3.11	13.280	3.48	14.106	3.68	14.255	3.64
13.054	3.07	13.153	3.25	13.282	3.37	14.110	3.55	14.259	3.54
13.056	2.87	13.155	3.34	13.284	3.36	14.112	3.66	14.263	3.68
13.058	2.75	13.157	3.40	13.318	3.17	14.114	4.84	14.267	3.04
13.060	3.62	13.159	3.00	13.320	2.93	14.115	3.24	14.276	3.47
13.062	3.15	13.161	3.30	13.322	3.22	14.117	4.29	14.280	3.56
13.064	2.76	13.163	2.70	13.324	3.12	14.118	4.03	14.283	3.60
13.065	2.81	13.165	3.22	13.326	3.05	14.120	3.76	14.287	3.53
13.067	2.67	13.167	3.64	13.328	3.15	14.122	3.78	14.291	3.11
13.069	2.12	13.169	3.25	13.330	3.03	14.123	3.51	14.295	2.28
13.071	2.61	13.171	3.12	13.332	3.13	14.124	3.38	14.300	3.58
13.073	3.11	13.173	2.80	13.334	2.85	14.126	3.69	14.303	3.48
13.075	3.42	13.175	2.71	13.338	3.02	14.127	3.89	14.306	3.29
13.077	2.72	13.179	2.46	13.340	3.00	14.128	3.66	14.310	2.85
13.079	2.53	13.183	3.39	13.342	3.03	14.130	3.62	14.314	3.60
13.081	2.75	13.187	3.31			14.131	3.56	14.318	3.51
13.083	3.06	13.191	3.53	14.001	0.99	14.132	3.56	14.323	3.15
13.085	4.11	13.195	3.02	14.006	2.94	14.134	3.67	14.326	1.51
13.087	2.87	13.199	2.77	14.008	3.56	14.136	3.47	14.330	3.49
13.089	2.65	13.203	2.60	14.010	5.83	14.138	3.83		
13.091	2.97	13.207	3.31	14.012	3.50	14.142	3.69		
13.093	2.87	13.211	3.01	14.014	3.51	14.146	3.75		
13.095	2.81	13.215	2.97	14.016	3.28	14.150	3.60		
13.097	3.02	13.219	2.44	14.017	4.19	14.154	4.24		
13.099	2.64	13.223	3.01	14.018	3.13	14.158	2.45		
13.101	2.75	13.227	3.32	14.020	2.89	14.164	1.01		
13.103	2.77	13.231	2.42	14.022	3.45	14.172	3.55		
13.105	2.77	13.235	3.05	14.024	3.55	14.174	3.57		
13.107	2.76	13.237	3.56	14.027	3.55	14.177	3.88		
13.109	2.90	13.239	3.51	14.029	2.63	14.183	3.94		
13.111	2.88	13.241	3.55	14.031	2.03	14.188	2.15		
13.113	2.85	13.243	3.42	14.033	5.05	14.194	1.57		
13.115	3.46	13.245	2.98	14.040	3.65	14.195	1.39		
13.117	2.99	13.247	3.27	14.043	3.53	14.199	1.54		

SIZE STRUCTURE AND GROWTH RATE OF *Euphausia pacifica* OFF THE OREGON COAST[1]

MICHAEL C. SMILES, JR.,[2] AND WILLIAM G. PEARCY[3]

ABSTRACT

Euphausia pacifica (Hansen) off Oregon has a maximum life expectancy of about 1 year. During this time it grows rapidly to a length of 22-24 mm. Furcilia larvae were found throughout the year but were most abundant during the autumn months. The population density and the proportion of juveniles was higher within 25 miles of the coast than in offshore oceanic waters.

Growth rates off Oregon are about twice those previously reported for this species from other regions. Spawning also appears to be later in the year. All these features may be explained by the high primary production which is extended throughout the summer by coastal upwelling and by the lack of wide seasonal fluctuations of water temperatures along the Oregon coast.

Euphausia pacifica is one of the most abundant euphausiids in the North Pacific Ocean. Dense populations are found in Subarctic and Transitional waters (Brinton, 1962a; Ponomareva, 1963) and off the Oregon coast (Hebard, 1966; Osterberg, Pearcy, and Kujala, 1964; Pearcy and Osterberg, 1967).

Euphausiids are important food for many marine carnivores (see Mauchline and Fisher, 1969, and Ponomareva, 1963, for reviews), and *Euphausia pacifica* is no exception. It is preyed upon by salmon (Ito, 1964), baleen whales (Nemoto, 1957, 1959; Osterberg et al, 1964), herring (Ponomareva, 1963), sardine and mackerel (Nakai et al, 1957, as cited by Ponomareva, 1963; Komaki, 1967), rockfish (Pereyra, Pearcy, and Carvey, 1969), pasiphaeid and sergestid shrimp (Renfro and Pearcy, 1966), pandalid shrimp (Pearcy, 1970), and myctophid fishes (Tyler, 1970).

Studies on the growth of several species of euphausiids are reviewed in the monograph by Mauchline and Fisher (1969). Data on the growth and life history of *E. pacifica* are limited. Nemoto (1957) presented some growth data for *E. pacifica* from the Japanese-Aleutian area. Ponomareva (1963), in her study on the distribution and ecology of euphausiids of the North Pacific, estimated the growth of *E. pacifica* from plankton samples collected during the winter and spring. Lasker (1966) determined the growth of *E. pacifica* reared in the laboratory. Preliminary growth rates of *E. pacifica* based on some of our data were also presented by Small (1967).

Because growth rates are needed to understand the ecology and energetics of a species, we undertook this study on the abundance, size structure, and growth rate of *E. pacifica* off Oregon.

COLLECTION METHODS

We made a total of 174 collections using 1-m mouth diameter plankton nets between June 1963 and July 1967 at stations located 15, 25, 45, and 65 miles off Newport, Oreg. In addition, 25 collections were obtained from stations 85-285 miles off Newport. These provided samples of *E. pacifica* for all seasons of the year over a 4-year period. Nets had 0.571-mm mesh openings and were used with a flowmeter placed in

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the mouth to measure the amount of water filtered.

The first 20 samples were from oblique tows, and the other 154 were from vertical tows. This change to vertical tows was made to ensure equal sampling at all depths throughout a tow. Comparison of the catches of several oblique and vertical tows taken during the same night indicated little difference in the number and size of *E. pacifica* per unit volume filtered.

Because euphausiids may avoid nets in the daytime, all tows were taken during nighttime when visual avoidance would be minimal (Brinton, 1967). This is also a period when *E. pacifica* presumably has migrated into the upper 100 m of the water column. *E. pacifica* captured in several 6-ft Isaacs-Kidd midwater trawls were measured to see if large euphausiids that were possibly avoiding the small vertical meter net could be captured. There was no indication that the maximum size of trawl-caught was larger than meter net-caught euphausiids.

The maximum depth of our tows was usually 200 m. Because Ponomareva (1963) suggested that *E. pacifica* adults inhabit the 200-500-m layer in their second winter and no longer migrate daily to the surface, tows were taken to 1000 m with both the midwater trawls and vertical meter nets. These deeper tows, however, did not contain any larger animals. Twelve vertical meter net samples from depths of 200 m or 1000 m to the surface did not show appreciable differences in size structure. Therefore, we assumed that a representative sample of the *E. pacifica* population was caught in the upper 200 m at night.

The entire plankton sample was preserved at sea in neutralized 10 % Formalin. In the laboratory ashore, all euphausiids were removed from each sample unless the number of euphausiids was large (more than 200 individuals). In such cases the sample was usually divided in half with a Folsom plankton splitter (McEwen, Johnson, and Folsom, 1954), and euphausiids were sorted from only one-half the sample. Males and females were not differentiated.

The length of each individual *E. pacifica* was measured to the nearest 0.1 mm from behind the eye to the posterior margin of the carapace, and each animal was then assigned to a 0.3-mm size-group. Total lengths (from the posterior of the eye to the tip of the telson) were also measured from randomly selected individuals of various lengths to enable comparisons of our data with those of others. A least squares fit of 146 comparisons gave the equation:

$$Y = 2.54\ X + 0.66$$

where Y = total length and X = carapace length. The variance was 248.19. Our measurements are all given as total lengths.

RESULTS

RECRUITMENT AND ABUNDANCE

Although larval *E. pacifica* occurred during almost all months of the year, definite trends in abundance were evident over the 4-year period (Fig. 1). Larvae were usually most abundant between October and December. During some years recruitment began as early as June and was also prominent in the summer months. No major concentrations of larvae were found during winter or spring.

These larval forms of *E. pacifica* were furcilia of about 7 mm or less, agreeing with Boden's (1950) size measurements and description of *E. pacifica* furcilia. Furcilia are found 16-18 days after spawning, usually within the upper 100 m of the water column (Ponomareva, 1963; Brinton, 1967).

Catch curves (Fig. 2) show the average number of different size-groups of *E. pacifica* collected during the entire study. All sizes of *E. pacifica* were much more abundant per m^3 inshore over the continental shelf than in oceanic offshore waters. Individuals larger than 15 mm were rare at station 65 miles or farther offshore. Our finding that larvae were less abundant at offshore than inshore stations agrees with Brinton (1962b), who also noted that *E. pacifica* was more abundant inshore than offshore of California. Thus, despite the wide oceanic distribution of *E. pacifica*, the density of near-

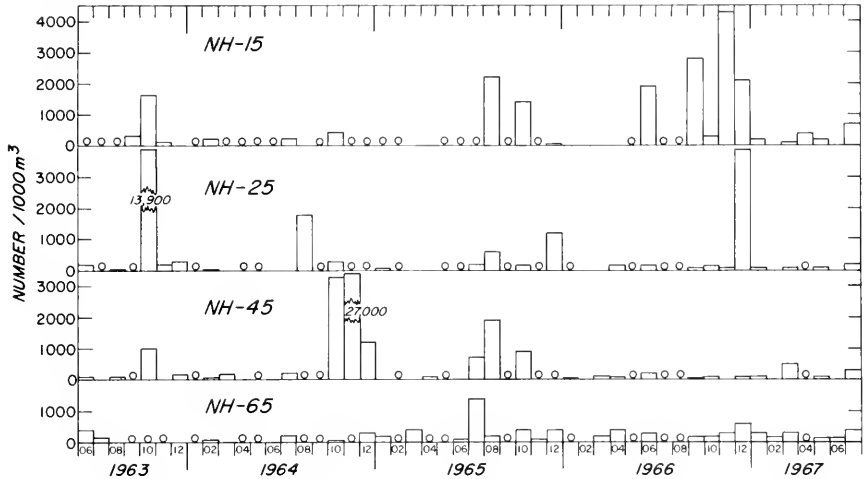

FIGURE 1.—Number of furcilia of *E. pacifica* collected at four stations off Newport, Oregon (NH-15, 25, 45, 65) during 1963-67. "0" indicates no sample taken for that month.

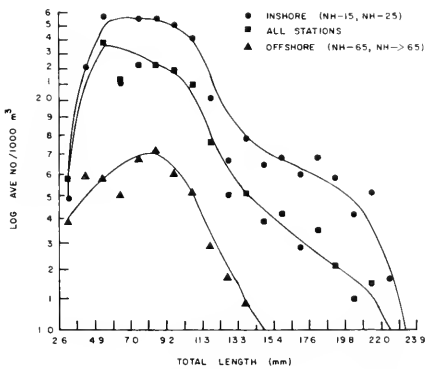

FIGURE 2.—Catch curves: the logarithm of the average number of various sizes of *E. pacifica* caught per 10^3 m^3 for all samples during the study.

shore populations may be considerably higher than offshore populations in the same region.

Although inshore tows were generally made only to 50 m and 130 m at the 15- and 25-mile stations respectively because of depth of water, euphausiid abundance at these stations was approximately 10 times greater than at offshore stations. This difference is too great to be explained by the differences in sampling depths even assuming that all euphausiids were concentrated in the upper 50 m at night.

GROWTH RATE

The extended spawning season and variability of catches of *E. pacifica* made interpretation of growth difficult. Three related methods, all based on progressions of size-frequency histograms, generally gave similar growth rates (Table 1) and led to the same conclusion: *E. pacifica* lives for a period of about 1 year and attains a maximum size of about 22-24 mm total length. We tenuously assumed for all these analyses that we sampled the same population, or populations with similar age structures and growth rates.

Two illustrations of growth based on monthly

TABLE 1.—Summary of average growth rate estimated from the progression of modes or means (see Figs. 3 and 4).

Year class	Recruitment month	Number months followed	Growth rates		
			Modes (Fig. 3 for 1965 and 1966 year classes)	Modes (Fig. 4)	Means
			Mm/month		
1963	09	10	1.6	1.9	1.6
1964	10	9	2.0	2.0	1.9
1965	10	8	2.1	2.2	2.0
1966	11	5	2.9	2.5	2.4
1967	03	3	2.6	2.5	2.5

size-frequency histograms of all stations combined (Fig. 3) illustrate the increasing modal lengths between December and June for the 1965 and 1966 year classes. Recruitment of small *E. pacifica* is also obvious during the spring of 1966 and 1967 and also shows a shift in modes with time. The 1963 and 1964 year classes (not shown here) showed similar trends.

A modified histogram plot (Fig. 4) was used to show the data for all 4 years and all 4 stations together. The advantage of this method is that one can follow the main modes of different sizes throughout the 4-year period. A disadvantage is that these plots are distorted by the arbitrary constraints that (1) at least 50 individuals per 10^3 m^3 of water within one size-group had to be present for plotting and (2) concentrations above $5000/10^3$ m^3 were plotted only as $5000/10^3$ m^3. All of the years represented in Figure 4 show some similarity. The main recruitment pulses are in the fall and summer, and the maximum size attained is approximately 22-24 mm length. After about 1 year, late in the second summer or fall, these large individuals disappeared from our collections. Interestingly, many of the modes that were composed of small euphausiids during the spring and early summer disappeared or were undiscernible by the fall. Either these individuals were subjected to higher mortality than the fall recruits or were transported out of the area. Apparently they made no major contribution to the local adult population.

Average lengths of size modes were also calculated for each collection using the computer techniques described by Hasselblad (1966). The means were generally close to the values for the modal lengths of various collections shown in Figures 3 and 4 and, therefore, are not illustrated here but are given in Table 1.

Our estimates of the growth of *E. pacifica* by all these methods are summarized in Table 1. As expected, estimates are similar for the same year classes. Growth varied from 1.6 to 2.9 mm per month among year classes, averaging about 2.0 mm per month. Growth rates were fastest for young stages. Year-classes 1963 and 1964 had slower average rates (1.6 and 2.0 mm/month) and were calculated over a longer period. Year-classes 1966 and 1967, on the other hand, were represented for the shortest periods of time and had the fastest average rates (2.9 and 2.6 mm/month). This deceleration of growth at the larger sizes is also apparent in Figure 3 where the growth rate from January to March 1966 was about 3.2 mm/month, while from March to June it was about 2.0 mm/month.

Our estimates are biased in several ways. They favored the recruitment pulses of the fall because the smaller modes of young that appeared earlier (June through September) did not comprise a good series of modal sequences. Moreover, the modes and means of the smaller sizes of *E. pacifica* are probably slightly overestimated since catch curves (Fig. 2) indicate escapement from our nets of individuals below 6 mm. This may cause an underestimation of growth rates.

DISCUSSION

Generalized growth curves of *E. pacifica* for three regions of the North Pacific are contrasted in Figure 5. On the basis of bimodal size-frequency distributions of winter and spring samples, Ponomareva (1963) concluded that *E. pacifica* lives for a period of 2 years. She found predominantly 8 and 14-15 mm individuals in the winter and 12-13 mm (her 1-year olds) and 19 mm (2-year olds) in the spring. Off Oregon not only were 12-13 mm individuals rare or absent in spring samples, but also 13-14 mm individuals, the size that Ponomareva would expect to find in the summer and fall, were absent. Moreover, our data, unlike Ponomareva's, show no large seasonal fluctuations of growth with retarded growth of the 13-14 mm sizes

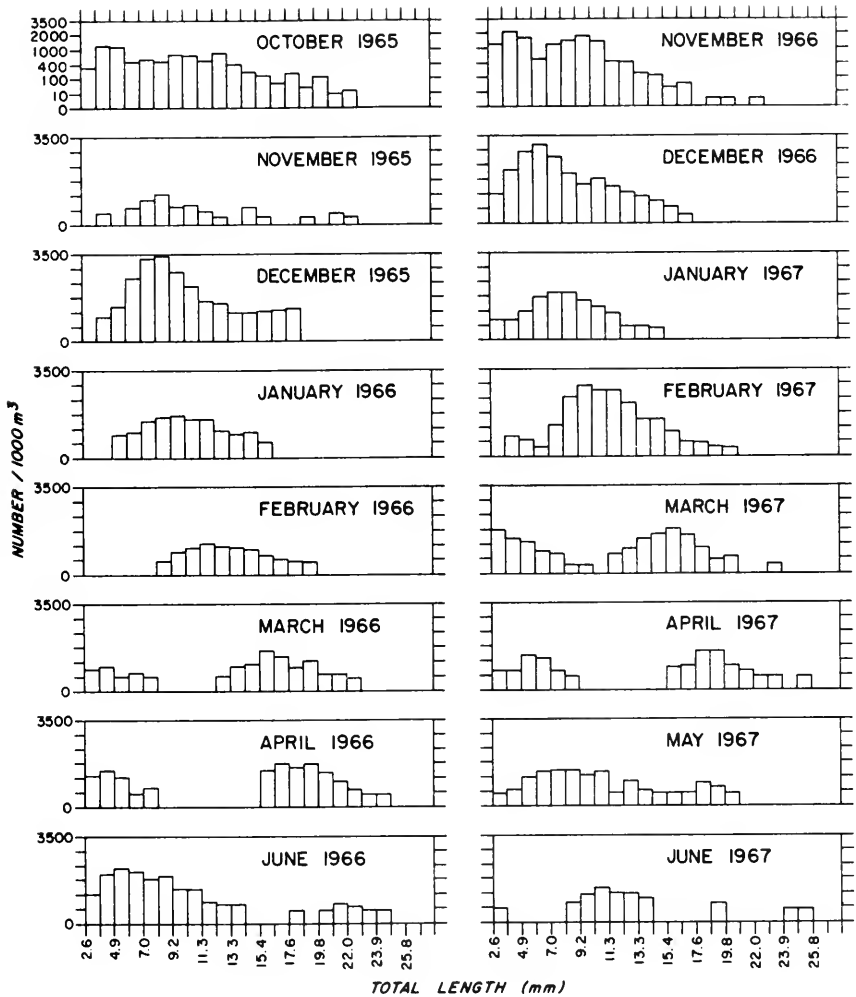

FIGURE 3.—Size frequency distributions of *E. pacifica* from all stations for the 1965 year class (left) and the 1966 year class (right).

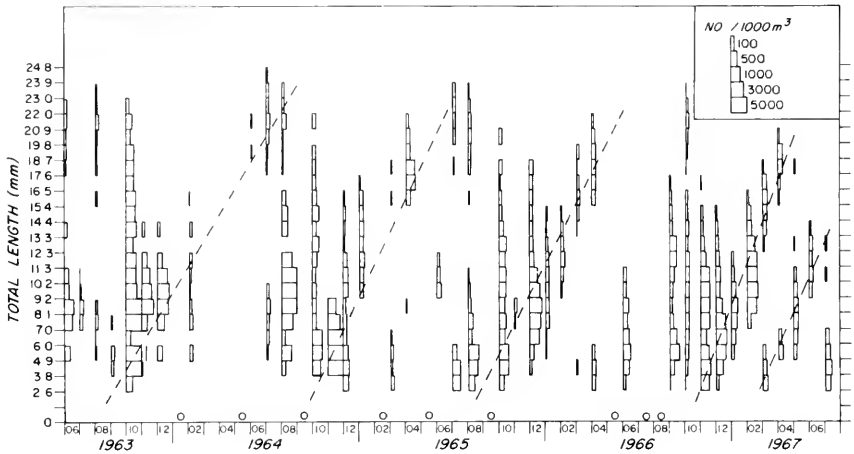

FIGURE 4.—Size frequency histograms for all stations, 1963-67. Dashed lines are an estimate of average growth of individual year classes. "0" indicates no samples for that month.

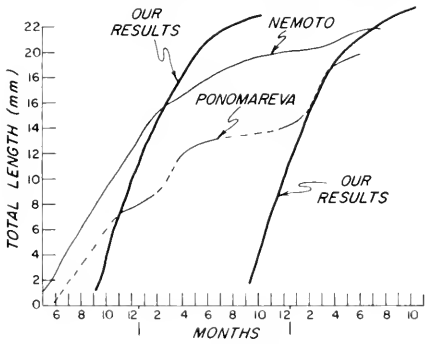

FIGURE 5.—Comparison of generalized growth curves of *E. pacifica*.

in the summer and fall. Nemoto (1957 and personal communication) believes that *E. pacifica* grows rapidly, reaching a length of 17-18 mm after 1 year. Many individuals spawn after 1 year and then may continue to live for another year, reaching a maximum of 22 mm after 2 years. We find no convincing evidence, however, for continuation of large adults through a second year. Large euphausiids disappeared from our samples by the winter (Fig. 4).

Thus our results indicate a faster growth rate and shorter life cycle than those of Ponomareva and Nemoto for the northwestern Pacific but a similar maximum size. Our growth rates off Oregon averaged 0.065 mm/day for the entire life span, about twice those for the other field studies of *E. pacifica*. Maximum rates for rapidly growing juveniles were 0.095 mm/day. These rates are higher than Lasker's (1966) maximum rates for juvenile *E. pacifica* reared in the laboratory, suggesting that growth in nature may exceed "optimal" conditions in the laboratory.

Although our estimates of the growth of *E. pacifica* are higher than previously reported, they approximate the estimates for several other species of euphausiids. A length of about 22 mm after 1 year was also found by Mauchline (1966) for *Thysanoessa raschii;* by Ruud (1936), Mauchline (1960), and Einarsson (1945) for *Meganyctiphanes norvegica;* by Einarsson (1945) for *Thysanopoda acutifrons;* by Ruud

(1932), Bargmann (1945), and Marr (1962) for *Euphausia superba;* and by Baker (1959) for *Euphausia triacantha*. Most of these species have a maximum life expectancy of 2 years, reproduce each year, and grow slowly during the winter. Other species are known to have a life expectancy of 1 year (Mauchline and Fisher, 1969).

Development, growth, and sexual maturity of the same species of euphausiid are known to vary among geographic populations (Einarsson, 1945; Nemoto, 1957; Ponomareva, 1963; Mauchline and Fisher, 1969). Mauchline and Fisher (1969) stress that this variability is probably directly related to differences in food and temperature. Hence, the rapid growth of *E. pacifica* off Oregon may be related to the high productivity of the region and the lack of large seasonal temperature fluctuations in nearshore waters.

Small, Curl, and Glooschenko[4] report high values for primary productivity in the coastal waters off Oregon. Curl and Small[5] found that standing stocks of chlorophyll-*a* averaged highest inshore and steadily decreased offshore. High production and stocks persist through the summer, the upwelling season, in inshore waters, whereas offshore waters have a typical summer productivity minimum (Anderson, 1964). Note that those seasonal and inshore-offshore gradients in phytoplankton are correlated in time and place with the spawning of *E. pacifica* off Oregon, mostly inshore and protracted over the summer and fall months. Ponomareva (1963) believes that phytoplankton is not only important as food for euphausiid larvae, but also may be necessary in the diet for development of reproductive products of *E. pacifica*.

Water temperatures along the Oregon coast are fairly uniform throughout the year and lack the extremes found along the eastern coasts of continents at similar latitudes. Advection of cool water to the surface (upwelling) during the summer and warm water toward shore during the winter moderates the usual seasonal variations. Pattullo, Burt, and Kulm (1969) observed that the seasonal range of heat content was twice as large offshore as inshore (within 65 miles) of the Oregon coast. The absence of severe winter temperatures may help to explain the rapid growth of *E. pacifica* throughout the year off Oregon. Conversely the slow and seasonally variable growth of *E. pacifica* found by Ponomareva (1963) was in the Far Eastern Seas of Asia where temperatures are often lower and where thermal variations are greater. The fact that *E. pacifica* is the only widespread euphausiid that spawns in the summer, when the phytoplankton bloom was almost over, indicates that this boreal species may be poorly adapted to the cold marginal Far Eastern Seas (Ponomareva, 1963).

The main pulses of larvae, hence spawning, of *E. pacifica* were in the fall, and not in the spring and summer as found by Ponomareva (1963), Nemoto (1957) off Japan, and Barham (1957) in Monterey Bay, Calif. Brinton (personal communication) notes larval recruitment throughout the year off Southern California. The later spawning off Oregon, like the rapid growth, may again be related to the prolonged production cycle caused by upwelling off Oregon and the moderate fall and winter water temperatures.

[4] L. F. Small, H. Curl, Jr., and W. A. Glooschenko. Seasonal primary production in a region of upwelling. III. Effects of solar radiation and upwelling on daily production. Unpublished MS.

[5] H. Curl, Jr., and L. F. Small. MS.

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ESTIMATING PHYTOPLANKTON PRODUCTION FROM AMMONIUM AND CHLOROPHYLL CONCENTRATIONS IN NUTRIENT-POOR WATER OF THE EASTERN TROPICAL PACIFIC OCEAN[1,2]

WILLIAM H. THOMAS[3] AND ROBERT W. OWEN, JR.[4]

ABSTRACT

Previous work has shown that nitrogen is the limiting nutrient in poor (nitrate-free) water in the eastern tropical Pacific Ocean and has suggested that ammonium is the principal nitrogen source for phytoplankton in this water. Enrichment and uptake experiments with various concentrations of ammonium have provided values for the half-saturation constant, K_s, and the maximum growth rate, μ_{max}, which can be used to calculate actual growth rates with the hyperbolic model relating growth rate to limiting nutrient concentration. At two stations, growth rates calculated from ammonium concentration agreed well with those calculated from chlorophyll and ^{14}C production, and the hyperbolic equation could be combined with that using production and chlorophyll to calculate production alone. In this paper calculated production rates are compared with those observed from ^{14}C uptake measurements for a number of EASTROPAC cruises. The regression between calculated production and observed production is highly significant and the slope is close to 1.0, indicating reasonable agreement, particularly when all of the errors in the calculation, especially in K_s, are considered. The results suggest rather close control of phytoplankton production by the limiting nutrient, ammonium, in these near-surface, nutrient-poor waters.

This paper describes how concentrations of a limiting nutrient in sea water and some measure of the standing crop of phytoplankton can be used to estimate phytoplankton production. Estimated production is compared with observed ^{14}C production, and the two sets of values are shown to agree reasonably well when all the errors in the estimation are considered.

The EASTROPAC Expedition series has delineated particularly well areas that are rich in nutrients and that are nutrient-poor in the eastern tropical Pacific Ocean. Rich areas include the Peru Current, the Costa Rica Dome, and an area of equatorial upwelling extending across the EASTROPAC area (from the American coast to long 119° W). Poor areas lie to the north and south of the equatorial upwelling zone and to the west of the Peru Current and Costa Rica Dome. Rich and poor near-surface waters were mapped in previous papers (Thomas, 1969, 1970b) and will be shown in detail in the EASTROPAC Atlas (Thomas, unpublished data). Nutrient values for rich and poor water are also given in Table 1 of Thomas (1970a).

Corresponding areal and seasonal changes in the phytoplankton production in this region have been observed and attributed in part to mechanisms of nutrient supply (Owen and Zeitzschel, 1970). No accounting has been possible, however, for the variations observed within the nutrient-poor surface layer of the region.

Near-surface water in poor areas is especially low in nitrate-nitrogen; this nutrient is generally not detectable (<0.1 μg-at./liter). Ammonium-N is present in concentrations ranging up to 1 μg-at./liter and organic nitrogen can reach

[1] Contribution from the Scripps Institution of Oceanography.

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concentrations of 17 μg-at./liter, but this latter nitrogen source is probably not utilized by phytoplankton (Thomas, Renger, and Dodson, in press).

Prior to EASTROPAC (pre-1967) low nitrate/phosphate ratios in tropical Pacific poor water suggested that nitrogen was a limiting nutrient although ratios were increased when ammonium was included along with nitrate, and it was suggested that this latter nutrient alleviated N deficiency (Thomas, 1966).

Recent EASTROPAC enrichment experiments provided direct evidence for N limitation. Phytoplankton growth occurred in experiments where nutrients were added singly to sea water samples only with N addition, and if N was deleted from an otherwise complete enrichment, little or no growth resulted (Thomas, 1969, 1970b). The fact that photosynthetic assimilation ratios were only slightly (but significantly) decreased in poor water as compared with rich water testified further to the alleviation and control of deficiency by ammonium (Thomas, 1970a).

Having established which nutrient is commonly limiting, one can use a quantitative nutrient requirement in an appropriate mathematical model to estimate growth rates (production) from concentration of the limiting nutrient. Recent work (Caperon, 1967; Dugdale, 1967) indicates that the best model is hyperbolic:

$$\mu = \mu_{\max} \left(\frac{S}{K_s + S} \right) \qquad (1)$$

where μ is the phytoplankton specific growth rate, $\mu_{\max}$ is the maximum rate which is unlimited by low nutrient concentration, S is a measured limiting nutrient concentration in sea water, and K_s is the "half-saturation constant" —a nutrient concentration that supports a rate equal to $\mu_{\max}/2$. This equation is equivalent to the Michaelis-Menton formulation for enzyme kinetics and was first applied to bacterial growth rates by Monod (1942). Many biological processes follow the hyperbolic model and since growth is the result of a series of coupled enzymatic reactions, the hyperbolic model is the model of choice.

A previous paper (Thomas, 1970b) provides information on $\mu_{\max}$ and K_s (for ammonium) from which μ can be calculated. To obtain these values we enriched samples of nutrient-poor Pacific sea water from a depth of 10 m with a complete mixture of non-nitrogenous nutrients to which various concentrations of ammonium were added. The samples were then incubated in natural light approximating the intensity that would be found at 10 m depth. Growth was estimated by successive daily measurements of *in vivo* chlorophyll (Lorenzen, 1966) in each treatment, and rates integrated over a daily period were calculated from the maximum increases in chlorophyll. These rates were plotted against ammonium concentrations to fit a hyperbolic model and values of K_s and $\mu_{\max}$ were obtained from the plot. These values and their 95% confidence limits are given in Table 1 for two such experiments. K_s values can also be determined from uptake experiments since recent work has shown that K_s values for growth and uptake are equivalent (Eppley and Thomas, 1969). Also included in Table 1 are uptake K_s values obtained by MacIsaac and Dugdale (1969) for nutrient-poor tropical Pacific water. Their values for $V_{\max}$, the maximum uptake rate, are not equivalent to $\mu_{\max}$ and thus are not included

TABLE 1.—Rate parameters for growth and uptake on ammonium in nutrient-poor tropical Pacific sea water.

Cruise	Station	K_s (μM)	95 percent limits	$\mu_{\max}$ (Doublings/day)	95 percent limits	Reference
EASTROPAC 76	007	1.68	± 3.28	1.12	± 0.83	Thomas (1970b)
EASTROPAC 76	173	1.47	± 0.91	1.22	± 0.27	Thomas (1970b)
Thompson 26	15	0.10	--	--	--	MacIsaac and Dugdale (1969)
Thompson 26	36	0.55	--	--	--	MacIsaac and Dugdale (1969)
Te Vega 13	651-a	0.62	--	--	--	MacIsaac and Dugdale (1969)
Mean values		0.88		1.17		
95 % limits of mean		1.33		0.14		

in Table 1. It will be noted that confidence limits for K_s values in given experiments are large as is the confidence limit for the mean of all five values which is used in subsequent calculations (see Results and Discussion). This can be attributed to lack of precision in measuring either growth or uptake; even in controlled experiments with laboratory cultures, K_s values are imprecise (Eppley, Rogers, and McCarthy, 1969; Eppley and Thomas, 1969).

The integrated daily growth rate, μ, can also be calculated from ^{14}C production estimates and chlorophyll concentrations using the following equation:

$$\mu = \frac{3.32\ [\log_{10}(R \cdot \text{chl} + \text{Prod}) - \log_{10}(R \cdot \text{chl})]}{1\ \text{day}} \qquad (2)$$

as has been done for laboratory cultures by Thomas (1964) and McAllister, Shah, and Strickland (1964). In this equation R is the carbon/chlorophyll ratio; $R \cdot$ chl thus is the standing stock of phytoplankton carbon. The constant 3.32 converts logarithms to the base 10 to logarithms to the base 2 and allows μ to be expressed as doublings of phytoplankton carbon per day.

In the previous paper (Thomas, 1970b), μ calculated from ammonium (equation 1) was compared with μ calculated from ^{14}C production and chlorophyll (equation 2) for the two EASTROPAC stations where K_s and μ_{max} were determined from enrichment experiments. At station 76.007, μ calculated from ammonium was 0.385 doublings/day while that calculated from ^{14}C uptake and chlorophyll was 0.365 doublings/day. At station 76.173 both values were identical—0.276 doublings/day. For the calculation we used an R value of 98, that found by Eppley (1968) for nitrate-free water off La Jolla.

This excellent agreement suggested that we could set equation (1) equal to equation (2) and solve for production as a function of ammonium and chlorophyll using K_s and μ_{max} as constants. The new equation thus derived is

$$\text{Prod} = \text{chl} \cdot R \left[\text{antilog}\left(\frac{\mu_{max}}{3.32} \cdot \frac{S}{K_s + S} \right) - 1 \right] \qquad (3)$$

This expression allows a direct comparison calculated and measured ^{14}C production (see Results and Discussion).

METHODS

Methods for determining K_s and μ_{max} were given previously (Thomas, 1970b; MacIsaac and Dugdale, 1969)—see also the previous section. Chlorophyll and production samples were taken from the depth of the 50 % light level, which was always in the upper mixed layer and varied from 9 to 16 m. This depth was determined by multiplying the depth at which the Secchi disc disappeared by 0.38. This factor employs the assumption that the Secchi disc disappears at 16 % of surface light intensity (Strickland, 1958).

Chlorophyll was determined in these samples by filtration on glass fiber filters, followed by 90 % acetone extraction of the filters, and measurement of fluorescence of the extract (Yentsch and Menzel, 1963; Holm-Hansen, Lorenzen, Holmes, and Strickland, 1965) using equations developed by Lorenzen (1966).

Simulated *in situ* production was measured by adding 20 μc $Na_2{}^{14}CO_3$ solution to the samples (Steemann Nielsen, 1952) and incubating them in a tubular shipboard incubator space in which natural light intensity was attenuated to 50 % of that incident. Incubation was started at noon and continued until sunset at sea surface temperature. Following incubation the samples were filtered through HA Millipore®[5] filters and their radioactivity assayed ashore by G-M counting of the filters. The ^{14}C solution was standardized by liquid scintillation counting and the efficiency of the G-M counter for these filters was determined by combusting some of these and measuring the evolved $^{14}CO_2$ with an ionization chamber. Daily uptake was determined by multiplying the activity by 2; we also corrected for the isotope effect by multiplying by 1.05. Darkened samples were incubated with illuminated samples and dark uptake was subtracted from light uptake. No cor-

[5] The use of trade names is merely to facilitate descriptions: no endorsement is implied.

rections for respiration by phytoplankton were made.

Ammonium was measured ashore in frozen samples from a depth of 10 m by the method of Richards and Kletsch (1964). Some labile amino-N which is probably available to phytoplankton is measured along with ammonium by this method.

RESULTS AND DISCUSSION

For the comparison of calculated and measured ^{14}C production, we have used samples from 10 m incubated at light intensities approximating those at 10 m to determine K_s and μ_{max}, and actual ^{14}C values from the 50% light level. We did this so that light intensities would not be a factor in the comparison—that is, light was presumed to be at saturating intensities but not inhibitory, which would be the case if surface samples had been incubated in the growth experiments and compared with surface production.

Ammonium was not determined at all production stations, and we selected those production values where data were available for

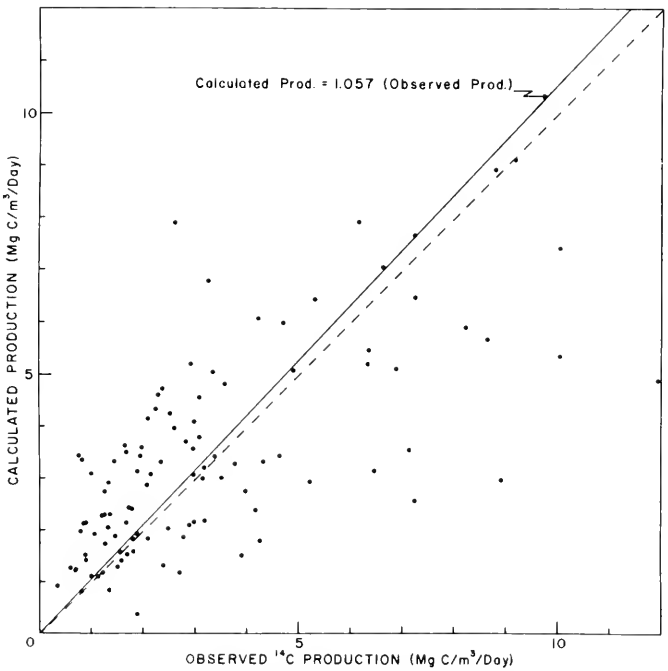

FIGURE 1.—Phytoplankton production calculated from ammonia and chlorophyll concentrations at 10 m compared with simulated *in situ* ^{14}C production at the 50 % light level in northerly nutrient-poor water in the eastern tropical Pacific Ocean. The dashed line is the regression that would be expected if agreement between the two sets of production values were perfect.

ammonium and where nitrate was undetectable. One hundred and five such production stations were available from 10 EASTROPAC cruises in this nutrient-poor water.

Production calculated from equation 3 is compared with measured ^{14}C production in Figure 1. There is a highly significant ($P<.01$) relationship between the two sets of values. The slope of the regression line is 1.057, which is very near to the value 1.0 which would be expected if agreement were perfect. Nevertheless, there is a large amount of scatter in the values of Figure 1; that is, the calculation overestimates in some cases and underestimates in others.

TABLE 2.—Errors in the calculation of production.

Parameter	Standard errors	Reference
Chlorophyll	± 12%	Halmes, Schaefer, and Shimada (1958)
R	± 17%	Eppley (1968)
μ_{max}	± 6%	Table 1 (this paper)
S	± 5%	Richards and Kletsch (1964)
K_s	± 76%	Table 1 (this paper)
Total	± 79%	
95% confidence limits	± 152%	

Errors in the values used in the calculation are given in Table 2. To figure total error these have been converted to variances and summed. The 95% confidence limit shows that any calculated production value can vary by ± 1.5 fold. Thus, one would expect quite a large scatter in Figure 1.

Most of the error is in K_s. When only the K_s values of Thomas (1970b) are used the calculation generally underestimates the observed ^{14}C production. Use of the mean of the K_s values of MacIsaac and Dugdale (1969) results in an overestimation. Since there is no reason to doubt either set of K_s values, we have used the overall mean K_s from Table 1. In applying this method to any other nutrient-limited waters, it would be well to obtain several values of K_s so that the error due to lack of precision in measuring K_s can be recognized.

Part of the scatter in Figure 1 may also be due to the fact that the parameter K_s is species—and temperature—dependent (Eppley, Rogers, and McCarthy, 1969) and that variations in species composition of the crop or slight variations in temperature may have affected the calculation. The parameters μ_{max} and R are also probably dependent upon the species composition of the crop and on temperature. Because of these factors, which are unknown, it is perhaps surprising that the relationship between calculated and observed production is so good when constant values of K_s, μ_{max}, and R are used.

This evidence supports the hypothesis that phytoplankton production in the upper mixed layer is controlled by the limiting nutrient, ammonium, and shows that the hyperbolic model describes this control very well. In this latter connection it should be noted that if a linear model having a term "S/S_{max}" in equation 3 (where S_{max} is that concentration supporting a maximum growth rate and which has a value near 10.0 μM from the data of Thomas, 1970b) is used rather than the term "$S/(K_s + S)$," the calculation very much underestimates the ^{14}C production. The linear model was used previously by Riley (1963) and Steele (1958) but should now be considered obsolete in view of more recent work using the hyperbolic model.

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ECOLOGICAL EFFICIENCY OF A PELAGIC MYSID SHRIMP; ESTIMATES FROM GROWTH, ENERGY BUDGET, AND MORTALITY STUDIES[1]

ROBERT I. CLUTTER[2] AND GAIL H. THEILACKER[3]

ABSTRACT

The net ecological efficiency (yield/assimilated) of a population of *Metamysidopsis elongata* (Crustacea, Mysidacea) is estimated to be 32 %. The gross ecological efficiency (yield/ingested) is probably between 19 % and 29 %.

Energy use by the field population was calculated from estimates of age specific natural mortality rates and data on growth, molting, reproduction, and respiration. Average growth and molting rates were determined by rearing the mysids in the laboratory. Size specific fecundity was determined from field and laboratory observations. The calorie contents of the mysids, their molts, eggs and larvae were estimated by bomb calorimetry and in part from biochemical composition. The energy used in metabolism was calculated from size specific respiration and data on body composition.

Biological systems are organized by the flow of energy. Trophic structure, numbers of steps in food chains, and numbers of conjunctions in food webs depend on the amount of energy passed through populations to other populations. Energy units provide a means of expressing productivity in terms common to all organisms.

The energy produced in the breakdown of biomass by organisms is stored as chemical energy in the pyrophosphate bonds of adenosine triphosphate (Morowitz, 1968). The overall thermodynamic efficiency of this process is similar in all animals, about 60 to 70 % according to Krebs and Kornberg (1957). It has been suggested (e.g. Slobodkin, 1961, 1962) that the efficiency of energy transfer between populations of animals is also fairly constant. This efficiency is necessarily of lower order because, for example, there are losses involved in synthesizing macromolecules, in continually resynthesizing proteins that undergo thermal denaturation, in transforming foodstuff energy into work energy (about 65 % efficiency), and in the degradation of energy during the performance of work. All energy that passes through a population is either lost as heat or passes on to another trophic level. If one assumes that all mortality is caused by predation, the gross ecological efficiency (Phillipson, 1966) of energy transfer through that population is the ratio of the energy yield in mortality to the energy ingested.

Through laboratory studies of growth, molting, reproduction, respiration, body composition, and energy content, we have constructed an energy budget for the pelagic mysid shrimp *Metamysidopsis elongata* (Holmes). Various aspects of the distribution, behavior, and population biology of this species have been described by Clutter (1967, 1969) and Fager and Clutter (1968). The energy budget data, together with estimates of natural population mortality rates, are used to estimate net and gross ecological efficiencies for the field population.

GROWTH AND DEVELOPMENT

Metamysidopsis elongata is a member of the Mysidae, a family that is ubiquitous and often very abundant in most of the neritic zones of the world ocean. This species is free-swimming and occurs in shoals and swarms just above the sand bottom in areas where surf is common

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(Clutter 1967, 1969).

As is characteristic of mysids, the eggs and larvae are held by the oostegites (brood pouch) of the adult females until they develop into juveniles that are similar in form to the adults. The juveniles grow by shedding their exoskeletons (ecdysis) at intervals that become progressively longer until they reach maturity. Males and females develop distinguishable morphological features during the period of rapid growth prior to maturity. Growth becomes progressively slower after maturity. Although there is no evidence that death occurs because of physiological aging, the maximum age observed was about 9 months. Most animals survive less than 3 months in the natural environment . We assume that most of the natural mortality is caused by predation, especially by fishes.

Some growth experiments have been reported for other species of Mysidae. Blegvad (1922) determined the growth rates of a few individuals of *Mysis inermis* from first stage juveniles through early maturity. Nouvel and Nouvel (1939) made disjunct determinations of time between molt stages for some size groups of *Praunus flexuosis*. Nair (1939) observed the time sequence in the egg and larva development of *Mesopodopsis orientalis*, determined the size and age at liberation, and noted the size at sexual maturity of males and females. In his review of growth in some marine Crustacea, Kurata (1960) presented the results of growth studies made by Ishikawa and Oshima on *Neomysis japonica* and by Matsudaira et al. on *Gastrosaccus vulgaris*. Mauchline (1967) maintained adult *Schistomysis spiritus* in the laboratory, estimated the time they take to attain sexual maturity, and estimated the minimum incubation time. Considering differences between species, sizes, and environmental temperatures, these reported patterns of development and size increase per molt are compatible with the results of our study.

CULTURE METHODS

Experimental animals were collected during the day from the middle of their habitat with nets (Clutter, 1965; Fager, Flechsig, Ford, Clutter, and Ghelardi, 1966). They were placed in large (20-50 liter), opaque plastic containers with covers and transported to the laboratory within 1 to 2 hr after the time of capture.

The culture methods were about the same as those described by Lasker and Theilacker (1965) for euphausid shrimps. Individual animals were placed in rectangular clear plastic containers in about 500 ml of sea water. The small containers were partly immersed in trays of running sea water. Since the running sea water was pumped continuously into the aquarium from midwater offshore, within the *Metamysidopsis* habitation zone, the laboratory temperatures (14°-20° C) were about the same as those that the animals would have experienced in their natural environment.

Animals of both sexes and of several sizes were selected for the experiments. Young juveniles were procured by placing pregnant females in containers and recovering the young on the day following their release from the brood pouch, which occurred at night. These young were then placed in separate containers. To determine the incubation time, i.e. the time from fertilization of the eggs to release from the brood pouch as juveniles, pregnant females with known times of fertilization were placed in individual containers so that larval development could be observed.

Mysids of all ages were fed freshly hatched nauplius larvae of brine shrimp, (*Artemia salina*). Twice each week the mysids were removed while their containers were emptied of excess food and cleaned with hot fresh water followed by a sea water rinse. They were then provided with excess quantities of fresh nauplii in clean sea water.

The containers were examined every day for the presence of molts or, occasionally, carcasses. The molts and carcasses were removed and placed individually in small vials of 5 % Formalin for subsequent microscopical examination and measurement.

OOGENESIS AND INCUBATION

Since *Metamysidopsis* has a transparent cara-

pace and body wall, it is possible to observe the late stages of oogenesis in live animals without dissecting them. The ovary (cf. Nair, 1939, for description) is situated in the interspace between the alimentary canal and the pericardial floor. Its most obvious feature is the pair of larger tubes that lay side by side. It is in these tubes that the eggs to be extruded into the brood pouch are invested with yolk. The process of yolk formation takes about a week in *Metamysidopsis* and is completed just before the female molts and copulates. By observing the ova in these tubes it is possible to estimate the size or age at first reproduction in maturing females, and to count the number of eggs that will be spawned by reproducing females of all ages.

Copulation occurs at night within 2 to 3 min after the mature female molts, during which time sperm are passed into the empty brood pouch by the attending adult male. The eggs are subsequently extruded into the brood pouch where they are fertilized. The eggs hatch from the vitelline membrane after 2 to 3 days. According to Manton (1928) and Nair (1939) a larval ecdysis occurs in the brood pouch shortly before the larvae are liberated. These late stage larvae have movable appendages and pigmented eyes that show through the transparent oostegites of the brooding female. The small quantity of yolk that is present after the larval ecdysis is absorbed, or nearly so, prior to liberation from the brood pouch.

After liberation the larvae tend to sink, then, according to Nair (1939), they undergo a second larval ecdysis after which the statocysts appear and they are capable of swimming. The mysids assume this highly mobile juvenile form within a few minutes after liberation. Although we did not attempt to distinguish sexes of larvae and juveniles, the observations of Nair (1939) indicate that dimorphism is exhibited by the antennules and abdominal appendages even though neither the brood pouch nor the penis is developed.

Incubation time was determined in the laboratory. Adult females and adult males were observed in an aquarium during molting and copulation. Ten females were caught after being observed *in copulo* and were placed in separate containers of sea water at the temperature of their natural environment at that time (17°-19° C). Five of them were removed, at various times, to determine the stages of development of the young. The remaining five all released their young as juveniles on the tenth day after fertilization.

In addition, a large number of nonpregnant adult females were kept in separate containers for various periods up to 157 days. The range of intermolt periods in 218 observations was 5 to 13 days; the median and modal values were both 10 days. There was no obvious temperature effect. The adult females molt just before fertilization and just after liberation of the young; therefore, the average incubation time was taken to be 10 days. This is intermediate between incubation times given for Mysidae that live and reproduce at higher and lower temperatures. Nair (1939) determined the incubation time of *Mesopodopsis orientalis* to be 4 days at 25° to 29° C. Mauchline (1967) reports a minimum incubation time of 3 weeks for *Schistomysis spiritus* at 12.5° C.

MOLTING

To avoid handling and possible injury of the experimental animals, the growth rates were determined by measuring molts. The molts suffered no appreciable decomposition because they were collected on the day following ecdysis. The morphological development of the animals was usually discernable from their molts. But the molts are fragile, split just back of the carapace where the animals emerge, and easily stretched out of shape. Therefore, to measure growth it was necessary to measure a part of the molt that always retained its form and bore a consistent relationship to the body length.

Uropod-Body Length Relationship

The exopod of the uropod (tail fan) was used to estimate the body length of each animal for its previous intermolt period. The uropods were measured from the base (end of last abdominal segment) to the tip, not including spines, which were sometimes broken, with an ocular micro-

meter, at 27.5 × magnification.

The relationship between uropod length and body length was established from a selected series of 94 animals that had been collected in the field and preserved. The series included animals that ranged in body length from 0.8 mm to 7.2 mm, and included late stage larvae, juveniles, immatures, and adults. Both sexes were included; there was no difference between sexes in this relationship.

The body length was measured from the end of the last abdominal segment (base of uropod) to the anterior edge of the carapace, behind the insertion of the eyestalk. Mysids tend to curl when preserved, and they can be distorted to appear longer if they are stretched when measured. To avoid this we chose specimens that were at most only slightly curved, and measured the length of the arc through the midline of those that had significant curvature, rather than the straight line distance between head and tail.

As shown in Figure 1, the relationship between uropod length and body length is linear. The body length is 4.5 times the uropod length.

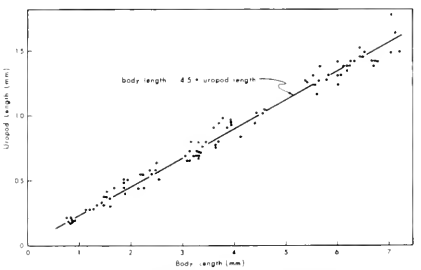

FIGURE 1.—Relationship between uropod length and body length of *Metamysidopsis*.

Molting Frequency

Average intermolt periods were estimated from 414 observations, 146 on males and 268 on females. In many cases several observations were made on the same animal. The maximum period of laboratory survival for a single animal was 157 days, and the maximum number of molts observed for a single animal (not the same animal) was 21. The molting frequency data for animals reared in the laboratory are summarized in Table 1. The sex of the juveniles was established after they had grown large enough to develop obvious morphological differences.

TABLE 1.—Frequency of molting periods observed for *Metamysidopsis* in the laboratory.

Sex and length	Intermolt Period (days)													
	3	4	5	6	7	8	9	10	11	12	13	Mode	Median	Mean
Females														
Body length (mm) 2		8										4	4	4.0
3		6	2									4	4	4.3
4		5	3	1								4	4-5	4.6
5		3	7	7	1	5	2					5-6	6	6.2
6			10	7	9	13	13	28	17	5	8	10	9-10	9.2
7			11	8	6	9	16	12	28	9	9	11	10	9.4
Males														
Body length (mm) 2	11											3	3	3.0
3	2	11	1									4	4	3.9
4	2	15	8	1	1							4	4	4.4
5		6	29	20	4							5	5-6	5.4
6		6	11	8	7	3						5	5-6	5.7

Supplementary data on molting frequency in the field population were obtained indirectly. Over a period of 3 days, 1,211 juveniles + immatures and 2,979 adults were brought into the laboratory late in the day and placed in large aquaria. The following morning all the animals and their molts were collected and counted. Of the juveniles + immatures 218 or 18 % had molted, and of the adults 356 or 12 % had molted. The reciprocal of the relative number molting is an estimate of molting period. The observed reciprocals were 5.6 for juveniles + immatures and 8.3 for adults. Since these values are midway in the ranges shown by laboratory animals (3-8 days for juveniles + immatures and 4-13 days for adults) we assume that the laboratory observations are valid estimates of molting frequency in the population as a whole.

Although our observations were made from February to October, and the water temperatures in the rearing troughs varied from 14° to 20° C, we were unable to detect any obvious effects of temperature or time of year on molting

frequency or growth rates. Nouvel and Nouvel (1939) stated that the intermolt period for *Praunus flexuosis* is least during the warmest months, and the incubatory period is 15 days in August and 3 to 4 weeks in September. Lasker (1966) showed that *Euphausia pacifica* intermolt periods varied as the water temperature fluctuated, and that the intermolt period was shortened by an artificially produced warm period, but that temperatures above 12° C did not accelerate molting further.

Since we do not have evidence to the contrary, we must assume that our laboratory observations on molting frequency provide adequate average values. From the median values given in Table 1 and estimated average growth rates (see below) we have estimated the molting schedules of females and males from juveniles to mature adults as follows:

Females: first six molts — 4 days
seventh molt — 5 days
eighth molt — 6 days
ninth molt — 8 days
tenth molt and thereafter — 10 days

Males: first four molts — 3 days
fifth to eighth molts — 4 days
ninth and tenth molts — 5 days
eleventh molt and thereafter — 6 days

GROWTH AND MATURATION

Evidence of the temporal sequence of growth and maturation can be obtained from following peaks of abundance of size groups in natural populations. We sequentially sampled the mysids in the field and observed some shifting peaks. But we consider that the results are not very reliable because of temporal changes in age-specific mortality rates (Fager and Clutter, 1968). Therefore, all the age-specific growth estimates presented here were obtained from laboratory studies.

Observed Growth

A few mysids were reared in the laboratory from fertilized egg to adult. Several were reared from egg through the juvenile stage. In addition, larger numbers of various sizes were collected in the field and kept in the laboratory for several molts.

The growth data from these animals were combined as shown in Figure 2 (females) and Figure 3 (males). The sexes were separated because the growth and molting rates of males and females are different. As they are shown in Figures 2 and 3, these individual growth curves are simplified and slightly incorrect representations of true growth, for two reasons. First, the growth of the body integument is represented to be continuous, whereas it actually occurs in discrete increments. Second, the age

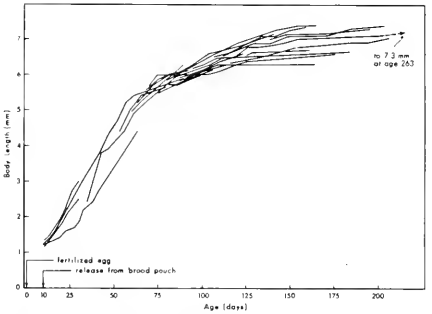

FIGURE 2.—Observed growth in length (from molts) of *Metamysidopsis* females in the laboratory.

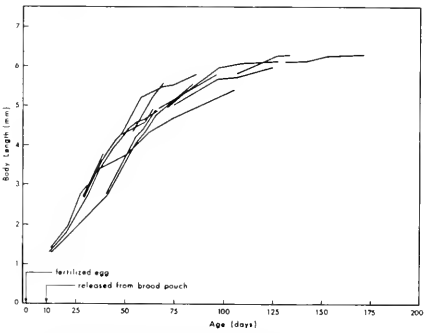

FIGURE 3.—Observed growth in length (from molts) of *Metamysidopsis* males in the laboratory.

shown is the age of the animal at the time it molted, rather than the age at the time that the molted integument was first formed. The procedure for combining the various growth curves of individual animals was to first plot the growth of the animals of known age, and then plot the other growth curves (actual ages unknown) in a manner that showed the least variation from the apparent trend.

Some of the apparent variability in growth rates may be attributable to differences in the temperature at which the growth occurred, but we did not detect any obvious temperature effect. Considerable individual variability occurred among animals of the same size or age that were reared simultaneously.

Maturation

Changes in morphology in relation to size, and known or estimated age, were observed in the molts of animals reared in the laboratory. Observations were made on live females collected from the field population to determine the size at which yolk invested ova first appear in the ovaries. Supplementary observations on the relationship between size and body form were made on preserved animals that had been collected in the field. There is some evidence from previous samples taken for other purposes (Clutter, 1967, 1969) that the relationship between size and stage of development may vary seasonally. But during the period of observations reported here, this did not appear to be significant.

In particular, we wished to determine (1) the size (and subsequently the age) at which males and females were easily distinguishable by their secondary sexual characteristics, (2) the size at the onset of maturity, and (3) the size at which spawning and brooding of eggs and larvae occurs. The external characteristics that most obviously separate males from females of this species are the enlarged oostegites (brood pouches) of the females and the enlarged pleopods (abdominal legs) and antennae of the males.

There is some variability in the size at which the stages of development occur. Therefore, our estimates are average values. The larvae are released and juvenile form is attained at age 10 days; at this time both sexes are about 1.2 mm long (body length; excluding antennae, eyes, and tail fan). Males exhibit sub-adult morphology when about 3.7 mm long, and become mature at 4.3 mm. Females exhibit subadult form at 4.0 mm, the ova become infused with yolk at 4.5 mm, and the eggs are extruded into the brood pouch, fertilized, and incubated at slightly less than 5.2 mm.

Average Growth in Length

Average continuous growth curves were fitted by eye to the combined growth data plotted in Figures 2 and 3. These curves are represented by the lower curves (fine, continuous unbroken lines) in Figure 4 (females) and Figure 5 (males). These continuous curves represent the size of the molt at the time—days from fertilization—that the molt was shed. Actually the integument of the animal had attained that size by the beginning of the intermolt period in question. The true growth of the integument of the average animal is represented by the stair-step pattern, which is based on the molting frequency analysis. The broken curved line of continuous growth (Fig. 4 and 5) represents the probable pattern of temporal change in average organic weight of the animal. This curve connects the points halfway between the beginnings and endings of the intermolt periods.

Since the average sizes at various stages of development were determined, it was possible to estimate the average time schedules of maturation and reproduction for females and males on the basis of the growth curves. The average female begins to develop a brood pouch at the seventh molt, 39 days after becoming a fertilized egg. Yolk invested ova begin to be formed at 45 days, during the ninth intermolt period; the ova are extruded into the developed brood pouch and fertilized at the beginning of the tenth intermolt period, at 53 days; and reproduction can occur at 10 day intervals thereafter.

Males and females grow at rates that are indistinguishable up to the age of about 30 days, even though the juvenile males molt more frequently than juvenile females. After that the

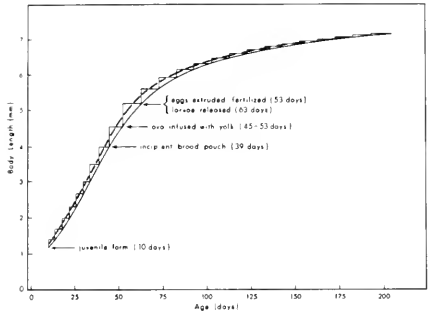

FIGURE 4.—Average growth in length of female *Metamysidopsis* in the laboratory. The lower curve (fine continuous line) was fitted to molt size data (Fig. 2). The steps represent changes in integument size. The upper curve (heavy broken line) represents the average size of the animals, assuming that the addition of body tissue is continuous.

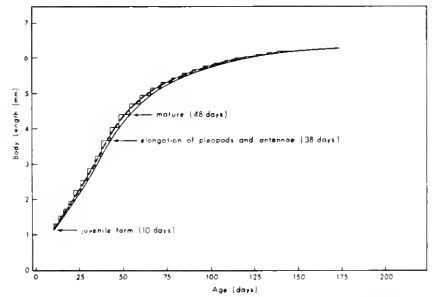

FIGURE 5.—Average growth in length of male *Metamysidopsis* in the laboratory. The lower curve (fine continuous line) was fitted to molt size data (Fig. 3). The steps represent changes in integument size. The upper curve (heavy broken line) represents the average size of the animals, assuming that the addition of body tissue is continuous.

males grow more slowly. The males develop easily recognized secondary sexual characteristics at an average age of 38 days and become sexually mature after about 48 days. Average age at maturity was estimated from observations of testes and copulatory behavior in the laboratory as well as from external morphology.

Average Growth in Weight

To estimate growth in terms of energy it is necessary to translate growth in length into growth in dry weight. This growth in dry weight is then translated into growth in organic (ash-free) weight and thereafter into calories.

The dry weights of *Metamysidopsis* of body lengths ranging from 1.9 mm to 6.5 mm were determined. The animals were captured alive, measured, washed very briefly with distilled water, and dried at 60° C in an oven for 24 hr. They were then weighed individually on a Cahn electrobalance immediately after they were removed from the oven.

The observed relationship between body length and dry weight is shown in Figure 6.

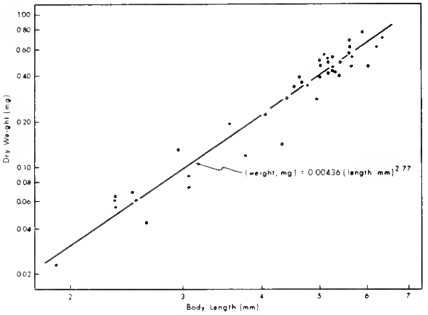

FIGURE 6.—Relationship between body length and dry weight of *Metamysidopsis*.

The equation for the relationship was determined empirically by fitting a straight line to the logarithms of body length and dry weight by the method of Bartlett (1949). The relationship is:

$$\log_e (\text{weight}) = -5.436 + 2.77 \log_e (\text{length})$$

or

$$\text{weight} = 0.00436\ (\text{length})^{2.77}$$

where weight is expressed in mg and length in mm.

It is common to assume that body weight and body volume have a linear relationship, and that body volume is proportional to the third power of length. Therefore dry weight is expected to

be proportional to the third power of body length (Bertalanffy, 1951). The observed relationship does not quite conform to the expected. The relationship between body length and body diameter appears to be linear (Fig. 7); therefore the body volume must be proportional to the third power of the body length. The observed relationship between weight and length could be the result of orthogonal growth of the appendages, which become progressively larger as the animals mature.

From the average length-weight relationship and the average continuous growth in length curves (Fig. 4 and 5) we have calculated the average growth in weight curves shown in Figure 8. The average continuous growth in length curves represented by the heavy broken lines in Figures 4 and 5 were used to calculate growth in weight, because we assume that growth in organic weight is continuous during intermolt periods even though growth of the integument occurs in discrete steps. The estimated growth in weight of males was extrapolated by eye from age 175 days to age 204 days. We do not have laboratory growth estimates for these larger males, but they occurred in the field population.

The average dry weight per egg (140 eggs in sample) was 5.5 μg. Larvae weigh slightly less than this because they lose weight through metabolism while in the brood pouch, even though their ash content is slightly higher than that of the eggs.

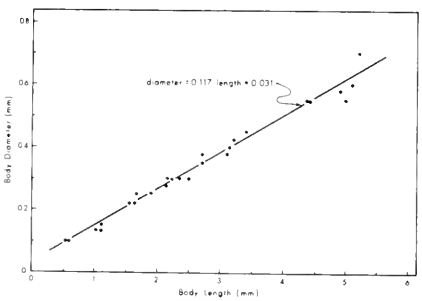

FIGURE 7.—Relationship between body length and body diameter of *Metamysidopsis*.

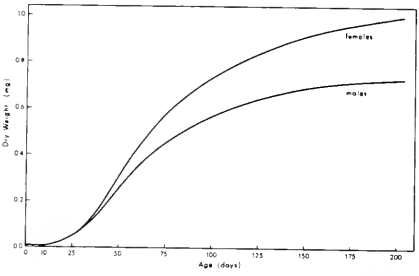

FIGURE 8.—Average growth in dry weight of *Metamydisopsis* females and males in the laboratory.

REPRODUCTION

Data on reproduction and associated energy use are easier to obtain for Mysidae than for most pelagic invertebrates. The eggs and larvae are carried in the brood pouch of the female, and the incipient eggs can be counted prior to their full development and extrusion because the body walls of the mysids are transparent. In addition, copulation and fertilization can be observed in the laboratory, and frequency of pregnancy among mature females can be observed in the natural population through sequential sampling because all stages live in the same area while gestating as they do when not reproducing. Nevertheless, average reproduction rate in these animals is not easy to assess with absolute certainty.

FECUNDITY

Minimum Estimate

The most straightforward way to estimate fecundity is to collect animals in the field, preserve them, and count the number of eggs or larvae carried by females of different sizes. Figure 9 shows the relationship between body lengths and number of young for 310 females collected in the field at various times during the year. The data include 125 females bearing eggs and 185 bearing larvae; we excluded animals that had obviously lost young during capture and preservation. For both eggs and lar-

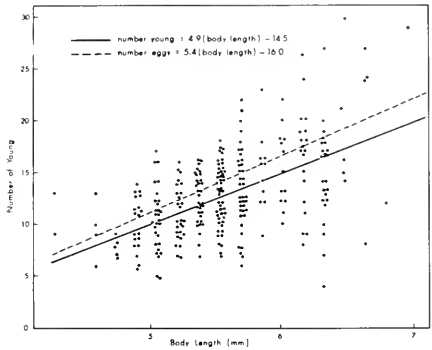

FIGURE 9.—Relationship between body length and number of brood pouch young (eggs and larvae) of preserved animals that were collected in the field. The lower line (continuous) was fitted to the points by the method of Bartlett (1949). The upper line (dashed) represents the equivalent relationship for newly laid eggs, assuming a brood pouch mortality of 0.013/day (see text).

vae, the number of young per female is highly variable. The average relationship between the size of the female and the number of young, calculated by the method of Bartlett (1949), is represented by the straight line: number of young = 4.9 (body length, mm) – 14.5.

This estimate of fecundity is not quite correct because it was made from counts of eggs and larvae that were a few days old. Some eggs and larvae apparently are lost from the brood pouch during the incubation period. Therefore, we adjusted the relationship to account for the mortality which occurs during the incubation period. To estimate the mortality during incubation, counts were made of the maturing ova in the ovaries of 40 adult females and counts were made of late stage larvae in the brood pouches of 27 females of the same size, collected at the same time. The ratio of mean number of larvae/mean number of ova was 0.90. The larvae were estimated to be 8 days old, giving an instantaneous mortality rate of 0.013/day.

The average age of the eggs and larvae from the 310 preserved females (Fig. 9) was estimated to be 7 days. Therefore, the relative survival of the young in the brood pouch between the time of extrusion of the eggs and the estimated average age at which they were counted (7 days) was estimated to be about 0.91. The number of brood pouch young per female was adjusted to the equivalent number of eggs extruded per female by multiplying the number of young by 1/0.91 = 1.10. The relationship (Fig. 9) then becomes: number of eggs = 5.4 (body length, mm) – 16.0, which is shown in Figure 9 as the upper, dashed line.

We consider this to be a minimum estimate of fecundity, because some females that had lost eggs and larvae from the brood pouches during collection and preservation were probably included, despite our attempt to exclude them.

Maximum Estimate

We observed that the females that had released young during the laboratory experiments had a higher apparent fecundity than those that were collected and preserved in the field. It is possible that there was some bias in selecting animals for the laboratory experiments, but we were not aware of any. The number of young released per female is plotted against the body length of the female for those 17 specimens in Figure 10. The average relationship between

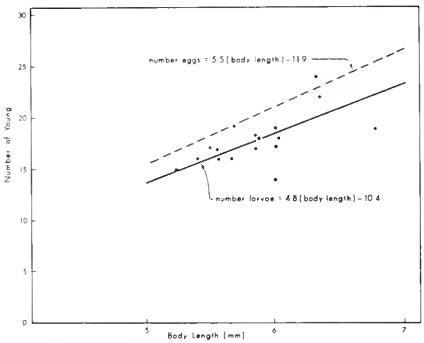

FIGURE 10.—Relationship between body length and number of young released by experimental animals in the laboratory. The lower line (continuous) was fitted to the points by the method of Bartlett (1949). The upper line (dashed) represents the equivalent relationship for newly laid eggs, assuming a brood pouch mortality of 0.013/day (see text).

body length and number of young, calculated by the method of Bartlett (1949), was: number of young = 4.8 (body length, mm) – 10.4. This is represented by the lower, unbroken straight line in Figure 10.

This relationship gives estimates of fecundity that are about 1.5 to 2 young per female higher than the relationship calculated from preserved animals. But this is not quite a maximum estimate of fecundity because it does not include the reduction from mortality that occurs during incubation.

As already demonstrated, we can assume a brood pouch mortality rate of 0.013 per day. The relative survival of young in the brood pouch during the 10 days between the extrusion of eggs and the release of larvae was therefore estimated to be 0.87. The number of young released per female was adjusted to the equivalent number of eggs extruded per female by multiplying the number of young by 1/0.87 = 1.15. The relationship (Fig. 10) then becomes: number of eggs = 5.5 (body length, mm) – 11.9, which is shown in Figure 10, as the upper, dashed line.

This relationship gives estimates of fecundity that are about four eggs per female higher than the minimum estimates calculated from preserved animals. We consider this to be the maximum estimate of fecundity. It is the same as that used by Fager and Clutter (1968).

COPULATION AND FERTILITY

The fecundity estimates given above apply only to the females that engage in copulation and are fertilized. Mature females that are not fertilized apparently extrude some eggs, but only about one-half the usual number.

Many observations of copulation were made in the laboratory (Clutter, 1969). It occurs in artificial light as well as in the dark, but only at night, between about 2000 and 2400 hr. It occurs within only 2 to 3 min after the mature females molt, and apparently only when the female exudes a pheromone to attract adult males of the same species.

Ten females were captured immediately after they were observed *in copulo* and kept in separate chambers for 10 days. Impregnation had been successful and the usual number of eggs were extruded in every instance. Some adult females that molt do not stimulate males to attend them. Ten adult females were captured after they had been observed to be unattended by males during molting and recovery. They later extruded only about one-half of the normal number of eggs, which eventually disappeared from the brood pouch, presumably because they were infertile. Therefore, the unfertilized females expended only about half the amount of energy in eggs that the fertilized females expended.

Since the mature females are subject to fertilization for only a few minutes following molting, and they apparently do not always attract males during the time, copulation does not always occur. Therefore, not all produce young every 10 days. In a large number of field collections during all seasons, the observed fraction of mature females carrying eggs or larvae in their brood pouches varied from 18 % to 78 %; the mean was 51 %. We are not certain of the source of this variability; there is some evidence that it could be related to population density (Clutter, 1969). We have assumed an average value of 50 % for the purpose of calculating the amount of energy used in reproduction.

On the average, mature females extrude the usual number of eggs about one-half of the time, and they otherwise extrude only one-half of the usual number of eggs. Therefore, the effective average fecundity, in terms of energy used in reproduction (but not in terms of the number of viable young produced), is 0.5 + (0.5) (0.5) = 75 % of the fecundity estimated from counts of young produced/female. For the purpose of calculating the amount of energy used in reproduction the fecundity equations are:

minimum — number of eggs = 4.1 (body length, mm) – 12.0

maximum — number of eggs = 4.1 (body length, mm) – 8.9

The second of these relationships is used in the ensuing energy budget calculations.

RESPIRATION

A polarographic oxygen electrode (Kanwisher, 1959) was used in a closed system to measure the respiration rates of *Metamysidopsis*. Both temperature and oxygen were recorded continuously on a strip chart.

The experimental animals were taken from large constant-flow holding tanks (temperature 14°-17° C) and acclimated overnight at the temperature used in the experiments (13.8°-18.1° C), to avoid the overshoot in oxygen consumption described by Grainger (1956). They were then washed in millipore-filtered seawater, counted, and transferred to previously filtered seawater in the oxygen electrode system. In each experiment an attempt was made to use animals of a limited size range. During the run they were held within a 10-ml chamber, baffled at each end with silk screen cloth of 282 μ mesh aperture size. The water in the closed system circulated through this chamber and then past the electrode at a constant rate. The whole system was immersed in a temperature-controlled water bath.

Oxygen use by bacteria was measured by making blank runs with the same water both before and after each test run. Bacterial use amounted to less than 2 %. Oxygen consumption by the mysids was corrected for bacterial uptake. The decrease in relative oxygen tension with time was nearly linear in both the blank runs and the test runs.

The results of the respiration experiments are shown in Table 2. Observed weight-specific

TABLE 2.—Summary of respiration experiments on *Metamysidopsis*.

Specimens	Number	Mean dry weight	Water temperature	Weight-specific respiration rate	
				Uncorrected	Corrected[1]
		Mg.	° *C*	*(μl O_2/mg dry wt hr)*	
Juveniles	99	0.03	13.8	7.71	7.54
Juvenile and immature males and females	176	0.07	18.0	5.40	4.76
"	297	0.08	18.1	5.08	4.48
"	297	0.08	18.1	6.78	5.93
"	132	0.14	13.8	3.92	3.82
Immature females	85	0.28	15.2	1.95	2.46
Males	51	0.31	13.8	3.60	3.53
Brooding females	27	0.47	13.8	3.22	3.16
"	27	0.66	13.8	2.65	2.59

[1] Corrected for oxygen saturation level and corrected to temperature of 16.0° C by using Q_{10} = 1.9 (Grainger, 1956).

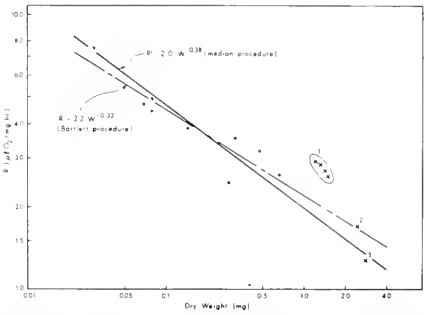

FIGURE 11.—Relation between respiration rate of *Metamysidopsis* and size at 16° C. The symbol R' represents respiration rate per dry unit weight (R/W). The lines were fitted to the circle points by two statistical pocedures. The x points are values calculated from published data on other species of Mysidae: 1-*Neomysis americana* (Raymont and Conover 1961); 2-*Neomysis integer* (Raymont, Austin and Linford 1966); 3-*Hemimysis labornae* (Grainger 1956).

respiration rates (μl O_2/ mg dry weight hr) were corrected for the initial percent oxygen saturation and for temperature. In correcting for temperature, a Q_{10} of 1.9 was used (Grainger, 1956). All values were corrected to 16° C, which is about the median of the year-round temperatures that occur in the natural environment of the mysids.

The corrected weight-specific respiration data are plotted in Figure 11 on log-log scales. The symbol R' (Conover, 1960) represents the respiration rate per unit dry weight (R/W). The average relationship between mean dry weight and R' was estimated by two statistical procedures. First, a straight line was fitted to the logarithmically transformed data by the median procedure (Tate and Clelland, 1957). This gave the relationship:

$$R' = 2.0\ W^{-0.38}$$

or

$$R = 2.0\ W^{0.62}$$

where R = respiration rate in μl O_2/hr
and W = mean dry weight in mg.

Second, a straight line was fitted to the logarithmically transformed data by the method of

Bartlett (1949). This gave the relationship:

$$R' = 2.2\ W^{-0.32}$$

or

$$R = 2.2\ W^{0.68}$$

Theoretically, the respiration rate is expected to be proportional to the $^2/_3$ power of weight. Since our estimates are slightly above (0.68) and slightly below (0.62) the expected value of 0.67, we consider that the $^2/_3$ power relationship is the best estimate for *Metamysidopsis* and that the best estimate of respiration rate (μl O_2/hr) is given by the equation:

$$R = 2.1\ W^{0.67}$$

Estimates of weight-specific respiration for three other, somewhat larger, species of Mysidae are compared with *Metamysidopsis* in Figure 11. The upper four points ("1" on Fig. 11) represents results for *Neomysis americana* from Raymont and Conover (1961) that were adjusted from 4° C or 10° C to 16° C by using a Q_{10} value of 1.6 that was estimated from their data. The intermediate point is an estimate of the median value oxygen consumption rate calculated from 12 determinations on *Neomysis integer* (Raymont, Austin, and Linford, 1966) that had been adjusted to 16° C by using a Q_{10} of 1.9 (Grainger, 1956). The lower point was estimated from the results of Grainger (1956) for *Hemimysis lamornae*. The ranges of values for these three larger species are about the same as the range (1-3 μl/hr) calculated from the seasonal change data of Raymont et al. (1966) that had been adjusted to 16° C. The estimates for *Metamysidopsis* and the other three Mysidae all lie well above the relationships calculated for marine planktonic Crustacea by Conover (1960).

BODY COMPOSITION AND ENERGY CONTENT

To estimate the amounts of energy used in respiration, molting, and reproduction it was necessary to determine the body composition of the mysids, their molts, and their young. For these analyses the animals were captured alive and, within 2 hr, placed in a constant-flow holding tank at 15° to 17° C where they were kept for a short time prior to analysis.

BODY COMPOSITION

The estimates of body composition of dried animals and molts are summarized in Table 3. The estimates for ash, protein, lipid, carbohydrate, and chitin are not considered to be accurate past the first decimal point. The fractional percentage values are entered so that the sums will equal 100 %. The methods by which these values were determined will be explained item by item.

To determine dry weights, the animals were washed very briefly with distilled water while still alive, then were oven-dried to constant weight at 60° C. Materials that were available only in small quantities were weighed on a Cahn electrobalance.

Ash

Ash content was estimated by incinerating

TABLE 3.—Average composition and energy content of dry *Metamysidopsis* bodies, molts, eggs, and larvae. Tabulated values for composition are %, and for energy content are cal/mg. The sums of % ash, "protein", lipid, carbohydrate and chitin = 100 %.

	Nitrogen	Carbon	Ash	"Protein"[1]	Lipid	Carbohydrate	Chitin	Energy
	%	%	%	%	%	%	%	*Cal/mg*
Body, whole	11.5	36.8	12.5	69.0	10.0	1.5	7.0	4.60
Body, organic	13.2	42.0	0	79.0	11.4	1.6	8.0	5.24
Molt, whole	--	23.5	44.8	30.9	0	0	24.3	2.46
Molt, organic	--	42.5	0	56.0	0	0	44.0	4.49
Egg, whole	--	58.0	6.0	35.2	58.8	0	0	7.16
Egg, organic	--	61.8	0	37.5	62.5	0	0	7.62
Larva, whole	--	45.7	6.6	60.8	28.9	0	3.7	5.78
Larva, organic	--	48.8	0	65.0	31.0	0	4.0	6.20

[1] "Protein" may include free amino acids.

whole animals or molts in a muffle furnace at 500° C and weighing the residue. Ash determinations were made on six samples composed of mixed animals, juveniles, immatures, adult males, and adult females. The samples contained from 2.7 to 7.3 mg of dried animals; the mean ash content was 12.5 % of the dry weight, and the range was 9.4 to 13.3 %. There was no obvious difference between age groups or sexes. This ash content is within the range, but slightly higher than the mean, of values reported for other Mysidae: *Mysis flexuosa* — 16 % (Hensen, 1887) and 11.9 % (Delff, 1912, quoted by Vinogradov, 1953); *Neomysis integer* — 7.9 % (Raymont, Austin, and Linford, 1964); *Siriella aequiremis* — 10.2 % (Omori, 1969).

Molts used for ash determinations were collected in the laboratory immediately after they were shed. Two samples, weighing 1.1 and 0.6 mg, composed of molts from a wide size range of mysids of both sexes had ash contents of 44.4 % and 45.7 %; the mean was 44.8 %. Lasker (1966) reported a similar value (46 %) for *Euphausia pacifica*. This high ash content in the molts suggests that a large fraction of the total body ash resides in the integuments of the whole animals. From 10 observations, we have found that the dry weight of the molt is on the average 13 % of the dry weight of the animal that sheds the molt. Assuming that the ash content of the molt is the same as the ash content of the integument of the whole animal, we estimate that 47 % of the body ash resides in the integument.

Ash content of brood pouch young was estimated from a large number of specimens taken from live females. A dry sample of 0.6 mg of newly hatched larvae had an ash content of 6.1 %. A sample of 1.2 mg of late stage larvae had an ash content of 6.6 %. Ash content of eggs was not determined; we assume that the ash content is slightly less than that of the newly hatched larvae, and we have used a value of 6.0 %.

Nitrogen and Carbon

Nitrogen content was determined by the micro-Kjeldahl method from three samples of mixed juvenile-adult animals. The dry weights of the samples were 12, 24, and 63 mg, and contained 13.1 %, 11.7%, and 11.2 % nitrogen respectively; the mean was 11.5 % of total dry weight. From a large number of determinations, Raymont et al. (1964) found a value of 11.4 % for *Neomysis integer*. Omori (1969) reported 11.0 % for *Siriella aequiremis*, and Jawed (1969) found 11.9 % for *Neomysis rayii*.

Carbon content was determined with an F and M carbon analyser model 180, described by Lasker (1966). We assume that all organic carbon, including that in chitin, is liberated by this method.

Three samples of females, without young, that weighed 0.2 to 0.4 mg, had carbon fractions between 35.6 % and 38.1 % of dry weight; the mean was 36.8 %. This estimate is intermediate among other values reported for mysids: *Lophogaster* sp. (family Lophogastridae) — 46.8 % (Curl, 1962a); *Neomysis integer* — 30.2 % and 29.5 % (Raymont et al., 1964, 1966); mixed mysids and euphausids — 40.7 % (Beers, 1966); *Siriella aequiremis* — 42.4 (Omori, 1969). From his analysis of several kinds of arthropods, Curl (1962a) found an average of about 38 % of the dry weight as carbon. He points out that this is about ¾ of the commonly assumed value of 50 % (Krogh, 1934).

In our carbon analysis of molts and young, we found that a 0.2-mg sample of fresh dried molts had 23.5 % carbon, a 0.4-mg sample of eggs had 58.0 % carbon, a 0.4-gm sample of midstage larvae had 47.1 % carbon. The carbon contents of the ash-free organic fractions of the material were calculated from these values. Lasker (1966) found 17 % carbon in the molts of *Euphausia pacifica* and 50 % carbon in the eggs.

Macromolecular Components

We assume that the body nitrogen of our species, *Metamysidopsis*, is present as protein, free amino acids, and chitin (Raymont, Austin, and Linford, 1968). We made no evaluation of chitin content, but used the value of 7 % determined for *Neomysis integer* by Raymont et al. (1964). The percent "protein" (may include free amino acids) was estimated by the following relationship, given that 16 % of "protein"

is nitrogen, 6.5% of chitin is nitrogen, and 7 % of the dry body is chitin: 0.16 ("protein") + (0.065) (0.07) = 0.115. From this relationship, the "protein" content of the whole dry body was estimated to be 69 %, which is similar to the value to 71 % protein estimated directly by Raymont et al. (1964) for *Neomysis integer*. According to the estimates of Raymont et al. (1968), the percent nitrogen in proteins of Mysidae may be lower than the value of 16 % commonly assumed for animal tissues. They found 13.3% N in the body protein of *Neomysis integer*, and estimated that about 17 % of what we would have designated as "protein" nitrogen was actually free amino acid nitrogen. They suggest that the amino acids may function in osmoregulation for *Neomysis integer*, which is a euryhaline-brackish water species. We know nothing directly about this for *Metamysidopsis*. Our species lives in a constant oceanic salinity, and we estimated the ash content to be higher than that of *N. integer*. Therefore, a high concentration of free amino acids may not be necessary for osmoregulation in our species. Whatever the ratio of protein/free amino acids may be in *Metamysidopsis*, our energy calculations should not be affected materially.

The lipid content of the mysid bodies was estimated by placing samples of dried, crushed bodies successively for 1 hr in each of two 10-ml portions of ethyl alcohol and two 10-ml washes of petroleum ether. The lipid content was estimated as the difference in dry weight before and after extraction. Two dry samples of mixed animals, weighing 62.9 mg and 13.4 mg, gave values of 9 % and 11 % lipid respectively. A third sample, containing 24.1 mg of brooding females that had full complements of young in their brood pouches, gave a value of 19 % lipid. Linford (1965) found that large females of *Neomysis integer* carrying young had higher lipid contents than males. From our knowledge of the number of young per female and the estimated percent lipid in the young, we calculate that ¼ to ½ of the 19 % lipid value could be contributed by the brood pouch young. Therefore, we have excluded the 19 % value from our estimate, and we have used 10 % as the estimate of average lipid content of the dry bodies. This is slightly less than the value of 13 % estimated for *Neomysis integer* by Raymont et al. (1964), but within the range of means for three species estimated from a large number of determinations by Linford (1965): *Mesopodopsis slavveri* — 9.0 %; *Neomysis integer* — 10.1 %; *Praunus neglectus* — 9.3 %.

The carbohydrate content of the mysids was estimated as the amount of macromolecular material remaining after the average estimates for ash, protein, chitin, and fat are subtracted from the dry weight. This remainder is 1.5 %. Apparently the carbohydrate fraction is low in all pelagic Crustacea. Raymont and Conover (1961) found that 1 % of the dry weight of *Neomysis americana* was glucose; Raymont and Krishnaswamy (1960) found 1.3 % carbohydrate in dry *Neomysis integer;* and Raymont et al. (1964) found 2.4 % carbohydrate in dry *Neomysis integer*.

We did no detailed analyses of the composition of molts, but we assume that the molt is composed of structural materials rather than energy storage materials. Since we consider that carbohydrates and lipids are virtually absent, we entered zero values for them in Table 3. The "protein"/chitin relationship was determined indirectly. First, we estimated the amount of carbon in the average protein of the mysids from the relationship:

$$(\%\ \text{C as protein}) = (\%\ \text{C in body}) - (\%\ \text{C as chitin}) - (\%\ \text{C as lipid}) - (\%\ \text{C as carbohydrate}).$$

The percent carbon in the organic fraction of the body is 42 %, the chitin fraction is taken as 8 %, the chitin is assumed to be 50 % carbon (Curl, 1962a), the lipid content of the organic fraction is 11 %, the lipid is assumed to be 77 % carbon (Lasker and Theilacker, 1962), the carbohydrate fraction is about 2 %, and the carbohydrate is assumed to be 40 % carbon (Curl, 1062a). Therefore, the percent carbon in the

mysid protein is calculated as:

$$\% \text{ C} = \frac{1}{0.79}[0.42 - (0.08)(0.50) - (0.11)(0.77) - (0.02)(0.40)]$$
$$= 0.364$$
$$= 36.4\ \%$$

This is considerably less than the average value of 52 % carbon in protein given by Hawk, Oser, and Summerson (1954), but similar to an estimate of 37 % made from the data of Lasker (1966), and higher than an estimate of 23 % made from the data of Raymont et al. (1964).

The second step in finding the relationship between chitin and protein in the molts was to estimate the chitin fraction from the following relationship:

(chitin fraction) (% C in chitin) + (protein fraction) (% C in protein) = (% C in molt)

where

chitin fraction + protein fraction = 1.0.

The chitin fraction calculated from this relationship is 44 % for the organic molt. The protein fraction is therefore estimated to be 56 %. This result suggests that a large fraction of the chitin may be reabsorbed by the animals before molting. This seems reasonable because in Crustacea the new endocuticle is formed during the intermolt period (between 2 % and 46 % of the time between molts, according to Passano, 1960).

To estimate the protein content of eggs and larvae, we have made some arbitrary assumptions that seem reasonable, and that do not measurably affect our energy calculations in any event. We have assumed that the eggs do not contain a measurable amount of carbohydrate, and that they contain little or no chitin because the integument is not yet formed. Therefore, we have assumed that the organic fraction of the eggs is either protein or lipid. For late stage larvae we have also assumed that carbohydrate is absent, but that some chitin is present because they form integument and molt once before they are released. We have assumed that the organic fraction of the larvae contains half the amount of chitin as the adults, or 4 %.

The protein-lipid composition of the eggs was calculated from the carbon content of the ash-free fraction. We have estimated (above) that 36.4 % of the mysid protein is composed of carbon, that 77 % of the lipid is carbon, and that 61.8 % of the ash-free egg is carbon. By using these values we calculate that the organic fraction of the eggs is 62.5 % lipid and 37.5 % protein. The carbon content of intermediate age brood pouch young (about 5 days old) was less than that of eggs and more than that of late stage larvae. For these intermediate age young we calculate a lipid content of 43 %.

ENERGY CONTENT

Juveniles - Adults

The ash-free calorie content of *Metamysidopsis* was determined in a Parr non-adiabatic calorimeter. The data, converted to ash-free values, are given in Table 4. Three of the samples contained so little material that Nujol supplement had to be added to raise the heat of combustion to a measurable level. All three of these measurements fell outside the 95 % confidence limits of the six determinations made without the Nujol supplement. The variability among the three supplemented determinations can be attributed to the ± 2 % variation of the caloric content of the Nujol supplement (10,791 ± 200 cal/g), because the weight of the supplement greatly exceeded the weight of the sample material in each case.

TABLE 4.—Ash-free[1] caloric content of *Metamysidopsis*.

Specimens	Dry weight	Calorie content
	Mg	*Cal/g*
Young juveniles	1.05	[2]3028.9
Juveniles	2.65	[2]6462.6
Young females	4.80	[2]4242.3
Advanced juveniles	12.55	5021.7
Immature males	17.30	5049.0
Immature males	17.30	5358.0
Mature males	15.75	5123.8
Mature females	12.40	5185.7
Mature females	17.25	5699.1

[1] Ash content 12.5 % used in all calculations.
[2] Nujol supplement used in determinations.

The mean for the six nonsupplemented samples is 5,240 cal/g (shown as 5.24 cal/mg in Table 3). No significant differences in energy content among developmental stages nor between sexes were found.

This mean calorie content estimate is somewhat lower than those reported for other crustacea. Slobodkin and Richman (1961) gave values of 5.4 to 5.6 cal/ash-free mg; Lasker (1965) reported a range of 4.9 to 5.4 cal/mg (including ash) for two species of copepods. Our mean value is also lower than the value that can be calculated from the information on body composition, together with reported average values of the calorie content of animal protein, fat, and carbohydrate. Conversion factors given by Morowitz (1968) are: protein, 5.5 cal/mg; fat, 9.3 cal/mg; and carbohydrate, 4.1 cal/mg. Since chitin is glucosamine, we have assumed that it, like carbohydrate, has a calorie content of 4.1 cal/mg. From these conversion factors and the composition data given in Table 3, we calculated an expected value of about 5.77 cal/ash-free mg.

We use the empirical value, 5.24 cal/ash-free mg, in our subsequent energy budget calculations. We consider this to be a conservative estimate, because it assumes that the mysid protein has an energy content of only 4.8 cal/mg. This lower than expected estimate may be related to the empirical observation that the mysid protein contains only 36 % carbon, rather than about 50 % as is commonly assumed for animal protein.

The juvenile and adult *Metamysidopsis* contained 12.5 % ash; therefore, the energy in the whole dry body of an adult or juvenile is estimated to be: (4.6 cal/mg) × (dry weight, mg).

Molts

We estimated the energy content of molts indirectly, because it was difficult to obtain enough material for calorie measurements. The ash-free fraction (55 %) of the molts was estimated to be composed of 44 % chitin and 56 % protein. By assuming that chitin has an energy content of 4.1 cal mg, and that the mysid protein has an energy content of 4.8 cal mg, we calculate that the ash-free fraction of the molts has an energy content of 4.5 cal/mg.

From a sample of 10 animals and their molts we found that the dry weight of molts is on the average 13 % (range 9-19%) of the dry weight of the animals that shed them. Lasker (1964, 1966) and Jerde and Lasker (1966) found that the dry molts of a euphausiid were about 10 % of the dry weight of the animals that produced them (range 4-14 %).

The energy lost by molting *Metamysidopsis* is therefore proportional to the size of the animal:

$$(0.13)\,(0.55)\,(4.5 \text{ cal/mg}) \times (\text{dry weight of animal, mg})$$

or

$$(0.32 \text{ cal/mg}) \times (\text{dry weight of animal, mg}).$$

Eggs and Larvae

We estimated that eggs were 6 % ash, 35 % protein, and 59 % lipid. The energy content of an egg is estimated to be: (0.35) (4.8 cal/mg) + (0.59) (9.3 cal/mg) = 7.16 cal/mg. A sample of 140 eggs was dried and weighed; the mean dry weight per egg was 0.0055 mg. The energy content per egg is therefore 0.039 calorie.

We estimated that, just before being released from the brood pouch, the larvae are about 6 % ash, 61 % protein, 29 % lipid, and 4 % chitin. The energy content of a late stage larva is estimated to be: (0.61) (4.8 cal/mg) + (0.29) (9.3 cal/mg) + (0.04) (4.1 cal/mg) = 5.78 cal/mg. The mean dry weight per larva, estimated from 110 individuals, was 0.0051 mg. The energy content per larva is therefore 0.029 calorie.

ENERGY BUDGET AND EFFICIENCY OF ENERGY TRANSFER

From the data on average growth, age-specific fecundity, respiration rate, and energy content we have calculated cumulative curves of energy use by individual mysids in attaining various stages of development. Data on age-specific natural mortality rates (Fager and Clutter 1968) were used to estimate l_x (probability

of animal being alive at age x) schedules and average generation time of the field population. The field and laboratory data were combined in an analysis of the efficiency of energy transfer through the *Metamysidopsis* population to the organisms that feed on them.

CUMULATIVE ENERGY CURVES

At age zero the egg contains about 0.04 cal. Ten days later, at the time it is released from the brood pouch, the larva contains about 0.03 cal. Thereafter the average calorie content increases in proportion to the dry weight (4.6 cal/mg). The average schedules of energy incorporation differ between males and females after about 30 days; the rate of incorporation becomes lower and levels off sooner in males. The accumulation of body energy is shown as the lowest curves in Figure 12 (females) and Figure 13 (males).

The amount of energy lost in molts varies with age because the size of the molt increases and the molting frequency decreases. Females and males have different cumulative losses of energy from molting because their growth rates are different after age 30 days, and their molting frequencies are different (Table 1.) Although the actual loss of energy in molting occurs at discrete intervals, we have plotted the cumulative energy loss as smooth curves, because the accumulation of energy for integument formation probably is continuous. Cumulative energy loss in molting is shown as the second curve in Figure 12 (females) and Figure 13 (males). The cumulative energy curves are additive, i.e. the area between the first curve (body energy) and second curve (molting energy) represents the cumulative energy loss in molts.

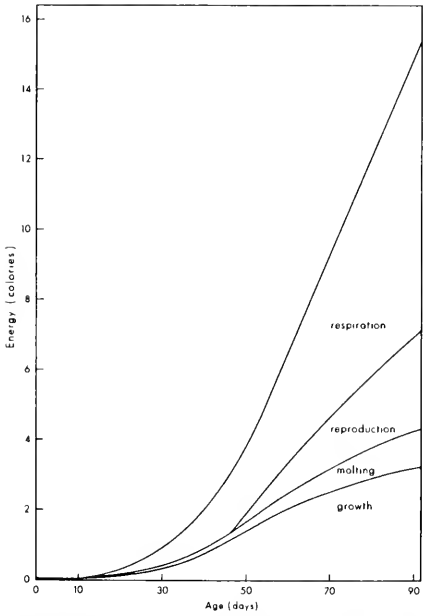

FIGURE 12.—Cumulative energy used by individual *Metamysidopsis* females. The curves are additive, i.e. the space between the lower two curves represents the cumulative energy lost in molts, the next higher space represents energy used to produce eggs (both fertilized and unfertilized), etc.—so that the upper curve represents cumulative energy used for all processes.

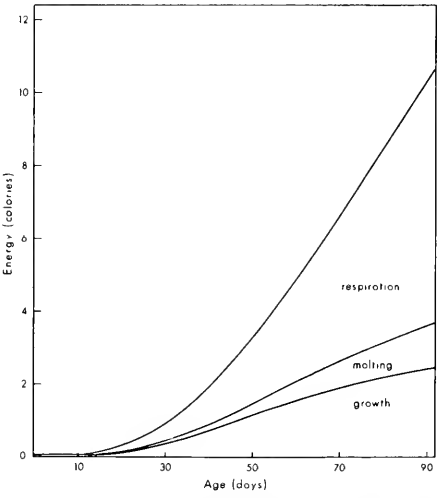

FIGURE 13.—Cumulative energy used by individual *Metamysidopsis* males. The curves are additive (see Fig. 12).

Males use a small amount of energy in producing sperm, but we assume that this is negligible. In females, the ova begin to be infused with yolk about age 45 days. The actual discharge of eggs occurs at discrete intervals of about 10 days, beginning at age 53 days. We assume that the accumulation of energy for reproduction is more continuous than this, therefore we have shown reproductive energy use as a smooth curve. The reproduction energy curve shown in Figure 12 is based on the maximum fecundity estimate given previously [number of eggs = 4.1 (body length, mm) — 8.9]. A reproduction energy curve based on our minimum estimate of fecundity [number of eggs = 4.1 (body length, mm) — 12.0] would be 0.12 cal (3.1 eggs) lower per spawning. This would make the minimum estimate 72 % of the maximum estimate at the age of first spawning (53 days) and progressively higher in percentage thereafter, e.g. 85 % at the age of fifth spawning (93 days). All our reproduction energy calculations take into account the observation that, on the average, mature females extrude the usual number of eggs only one-half of the time and otherwise extrude only one-half the usual number of eggs.

The amount of energy used in respiration was calculated from the weight-specific respiration equation: $R' = 2.1$ (dry weight, mg)$^{-0.33}$, and from energy conversion factors based on our estimates of body composition.

We do not know what substrate *Metamysidopsis* catabolizes. The organic fraction of the body is largely protein; the storage product (carbohydrate and lipid) content is low. Raymont and Krishnaswamy (1960) observed that the carbohydrate content of *Neomysis integer* decreased slightly, from about 1.30 % (of dry weight) to 1.06 %, when a marked reduction in feeding occurred. For the same species, Linford (1965) found no significant change in lipid level whether the animals were starved, fed a lipid-free diet, or fed a high lipid diet. Raymont et al. (1968) asserted that *N. integer* uses protein as an energy source.

We agree with Linford (1965) that it seems likely that the mysids must live largely on their daily ingestion. We think that the food they ingest has composition similar to their bodies. Therefore, our energy calculations assume that they use catabolic substrates in proportion to their presence in the body. This is supported by the results of Jawed (1969). To convert the amount of oxygen used in respiration into the equivalent energy lost as heat we have used the following values for calories lost/μl O_2 consumed (Hawk et al., 1954; Prosser, 1950): protein, 4.5×10^{-3}; lipid, 4.7×10^{-3}; carbohydrate, 5.0×10^{-3}. Therefore, our estimate of the average amount of energy used in respiration is about 4.5×10^{-3}cal μl O_2.

The cumulative energy used in respiration is shown as the uppermost curve in Figure 12 (females) and Figure 13 (males). The area between that curve and the next lower curve represents the catabolic heat loss. These respiration data were calculated for a temperature of 16° C, which was the median temperature of the natural environment of *Metamysidopsis*. Our respiration measurements were made in flowing water during the daylight hours. Therefore, they represent basal metabolism + energy expended in active swimming. There is some evidence (Clutter, 1969) that the mysids may be less active at night, even though they continue to swim at all times. For this reason we think that the field population may use somewhat less than this amount of energy in respiration.

Our estimated rate of energy loss in catabolism is higher than that estimated by Jawed (1969) in his study of nitrogen excretion in *Neomysis rayii*. He suggested that protein is catabolized in relatively large quantities, therefore nitrogenous excretion may provide a good estimate of catabolism. He found an average catabolism of about 2.5 % of body nitrogen per day in adult animals that were probably 8 to 10 mg dry weight, that were held at 10° C. The rate for adult *Metamysidopsis* of average size (0.6-0.8 mg) was 5 to 6 % of the body energy per day. This disparity in catabolism may result from differences between the size and between the environmental temperatures of the two species.

Jawed (1969) showed that about 15 % of the nitrogen was excreted as amino acids. We did

not investigate this in *Metamysidopsis*, therefore, our estimate of total catabolism could be slightly low because it includes only losses of heat energy.

NET ECOLOGICAL EFFICIENCY

Mortality and Generation Time

Estimates of natural mortality in the field population were made during the same period that the laboratory growth experiments were done (Fager and Clutter, 1968).

Brood pouch mortality rate was estimated to be 0.013/day (maximum of 0.017/day). Mortality rates for juveniles, immatures, and adults were estimated from consecutive series of field collections. The field mortality rates varied during the year. Survival curves (l_x = probability of being alive at age x) for periods of at least mortality, median mortality, and greatest mortality are shown in Figure 14. The mortality rates that we used to calculate these l_x curves are shown in Table 5. The greatest mortality rate results in a declining population; at the median mortality rate the population size remains about constant; and at the least mortality rate the population increases.

An average female first reproduces at about age 53 days. The generation length for the population is somewhat longer because the females reproduce more than once. The generation length for the field population varied between 67 days and 71 days; the median was 68 days (Fager and Clutter, 1968).

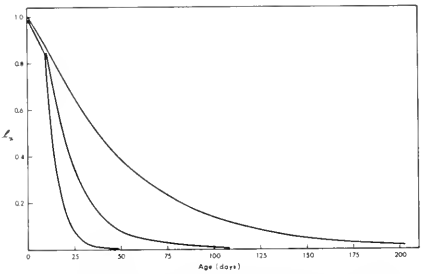

FIGURE 14.—Age specific survival (l_x = probability of being alive at age x) of *Metamysidopsis* calculated from estimates of greatest, median, and least mortality in the field population (Table 5).

TABLE 5.—Mortality rates (per day) used to calculate l_x schedules for the *Metamysidopsis* field population.

Specimens	Least mortality	Median mortality	Greatest mortality
Brood pouch young	0.013	0.013	0.017
Juveniles	0.02	0.06	0.15
Immatures	0.02	0.05	0.14
Adults	0.02	0.04	0.13

Relative Energy Use by Individuals

We determined the calories of energy used by average individual female and male mysids, and the fractions used for growth, molting, reproduction, and respiration from the estimates of cumulative energy use (shown in part in Figures 12 and 13). The amounts and the percentage distributions required to reach selected stages of development are shown in Table 6.

TABLE 6.—Energy used by individual *Metamysidopsis* to reach selected stages of development.

	Age	Energy	Relative use			
			Respiration	Reproduction	Molting	Growth
	Days	*Cal*	%	%	%	%
Females:						
Egg yolk production	45	2.7	52	0	8	40
First reproduction	53	4.6	49	9	7	35
Generation	68	8.7	50	15	7	28
$l_x = 0.01$	[1]103	18.4	55	19	7	19
Males:						
Maturity	48	3.0	54	0	10	36
$l_x = 0.01$	[1]103	12.8	67	0	12	21

[1] Approximate age at which $l_x = 0.01$ in a nearly stable population ($r \sim 0$).

The indicated age at which the probability of being alive reaches 0.01 applies to the stable population (median death rates).

The males require less energy to reach maturity than females, but relatively more of this energy goes into molting and respiration and less is incorporated. Two-thirds of the energy used in reproduction remains in the population; one-third is lost as unfertilized eggs.

The estimates of relative use of assimilated food by *Metamysidopsis* females during a life span are compared with estimates for a copepod and a euphausid (Corner, Cowey, and Marshall, 1967) in Table 7. The mysids apparently use a fraction of assimiliated energy for growth that is intermediate between the other two species, a lower fraction for metabolism, and a higher fraction for producing eggs.

TABLE 7.—Use of assimilated food by *Metamysidopsis* females (life span 103 days) compared with the copepod *Calanus finmarchicus*[1] (life span 10 weeks) and the euphausid *Euphausia pacifica*[2] (life span 20 months).

	Assimilated energy used by *Metamysidopsis*	Assimilated N used by *Calanus*	Assimilated C used by *Euphausia*
	%	%	%
Growth	19	25.3	10.1
Metabolism	55	61.4	72 3
Molts	7	0.9	16.6
Eggs	19	12.4	1.0

[1] From Corner, Cowey, and Marshall (1967).
[2] From Lasker (1966), revised in Corner et al.

Relative Energy Use by the Population

The values of relative energy use given in Tables 6 and 7 apply to individuals, or to populations wherein all members live a full life span. They do not apply to the natural population, because some die during all stages of growth.

We have estimated the relative amounts of energy that would be lost by populations in respiration, production of infertile eggs, molting, and mortality at the observed minimum, median and maximum mortality rates shown in Table 5. This was done by calculating the fraction of the population that died during each intermolt period ($\triangle l_x$), and multiplying this times: (1) the mean body energy content for the midpoint of that period, (2) the quantity of cumulative energy lost in infertile eggs up to the midpoint of that period, and (4) the quantity of cumulative energy used in respiration up to the midpoint of that period. The product values for each of these loss categories (mortality, molting, etc.) were then summed over all ages (to $l_x \sim 0.001$). The relative energy use values were calculated as fractions of the overall sum for all categories combined. We excluded fertilized eggs because this reproduction energy is retained in the population.

The age specific distribution of energy use (representing energy loss, because fertilized eggs are excluded) by a population (females and males) of *Metamysidopsis* at the median mortality rate is illustrated in Figure 15. All the curves are plotted with reference to the base line, zero. The rate of energy loss is low among eggs and larvae, and much higher among the juveniles that have just emerged from the brood pouch and begun to swim. In the larger animals, the respiration per unit weight is lower, but the respiration per animal is higher, so that the respiration rate per day is highest among the animals that are about 25 days old. The loss of energy per day from all causes is highest among the animals that are about 30 days old. After this the curve declines because the effect of larger size becomes less than the effect of smaller numbers.

The estimated relative amounts of energy lost by the population of females, males, and both sexes combined, for each loss category and

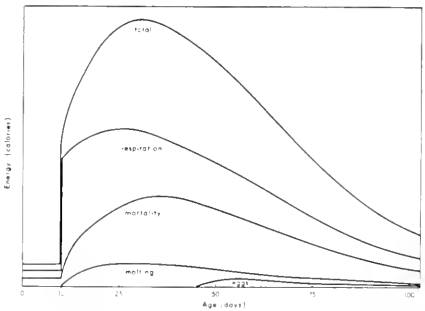

FIGURE 15.—Age specific distribution of energy loss by a *Metamysidopsis* population at the median mortality rate. Production of fertilized eggs is excluded.

for each of three mortality rates, are shown in Table 8. The percentages for females and males combined are not quite the same as the means of the separate percentages for females and for males. At the minimum death rate 55 % of the energy loss would pass through the female half of the population (58 % if fertile eggs are included). At the median death rate 52 % would pass through the females, and at the maximum death rate, 50 %.

TABLE 8.—Relative amount (%) of energy lost by *Metamysidopsis* populations in respiration, production of infertile eggs, molting, and mortality; at minimum, median and maximum mortality rates.

Sex	Death rate	Respiration	Infertile eggs	Molting	Mortality
		%	%	%	%
Females	minimum	63.7	6.7	8.6	20.9
	median	55.6	3.7	7.7	33.0
	maximum	45.4	0.1	6.1	48.4
Males	minimum	67.4	0.0	12.6	20.0
	median	58.3	0.0	9.9	31.8
	maximum	47.9	0.0	6.5	45.6
Females and Males	minimum	64.5	3.7	10.4	20.5
	median	56.9	1.9	8.8	32.4
	maximum	46.7	0.1	6.3	47.0

If we assume that all the mortality is yield to predators (Odum and Smalley, 1959; Engelmann, 1961), our mortality fractions are an estimate of net ecological efficiency (energy yield/energy assimilated). Apparently some Crustacea regularly die from natural causes other than mortality (e.g. *Daphnia*, Slobodkin, 1959). Many mysids of all ages died in our laboratory cultures, but we do not attribute this to senescence. In the field and in the laboratory we observed *Metamysidopsis* much older than the oldest animals that are involved significantly in our energy calculations. Our best estimate of the net ecological efficiency of the mysid population, for transfer of energy to a higher trophic level, such as fishes, is about 32 %. The net efficiency of transfer to all trophic levels is 1 — respiration fraction = 43 %.

ASSIMILATION AND GROSS ECOLOGICAL EFFICIENCY

Assimilation Efficiency

Gross ecological efficiency (energy yield/energy ingested) is the product of net ecological efficiency (energy yield/energy assimilated) × assimilation efficiency (energy assimilated/energy ingested). Therefore, an estimate of assimilation efficiency is required to estimate gross ecological efficiency for the mysid population.

We attempted to estimate the assimilation efficiency of *Metamysidopsis* directly by a carbon-14 method described by Lasker (1960). This failed because the mysids did not filter sufficient amounts of radioactive phytoplankton. An experiment with another member of the family Mysidae, taken from the same area, was successful. This gave an estimate of 90 % assimilation efficiency.

Lasker (1966) obtained a similar high value (84 %) for the morphologically similar *Euphausia pacifica;* and Marshall and Orr (1955) found values greater than 90 % for the copepod *Calanus finmarchicus*. In his detailed reviews of assimilation in zooplankton, Conover (1964, 1966) suggests that these values probably are too high. The very large number of observations, many of them his own, that are cited by Conover seem to be evidence that, although variable, the mean assimilation efficiency for crustacean zooplankton is at least 60 % and perhaps greater.

Gross Ecological Efficiency

From the information presently available we consider that the assimilation efficiency of the mysids is between 60 % and 90 %. Our best estimate of net ecological efficiency (yield/assimilated) is 32 %. Therefore, the minimum estimate of gross ecological efficiency (yield/ingested) is 19 % and the maximum estimate is 29 %.

These estimates are well within the broad range of available estimates of gross ecological efficiency (see reviews by Patten, 1959; Slobodkin, 1961; Phillipson, 1966; Reeve, 1966), and within the range of 8 % to 30 % that Engelmann (1961) considers to be acceptable. They are about 2 to 3 times as high as the median value of 10 % that is suggested by Slobodkin (1961, 1962), but lower than the values of 30 % to 50 % suggested for marine zooplankton by

Ketchum (1962), Steemann Nielsen (1962), and Curl (1962b).

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A LINEAR-PROGRAMMING SOLUTION TO SALMON MANAGEMENT[1]

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ABSTRACT

A linear-programming model was constructed to allocate the catch of salmon among the days of the salmon run. The objective of the model was to derive a management schedule for catching the salmon which would result in maximizing the value of the landings given certain constraints. These constraints ensured that cannery capacity was not exceeded, and that escapement of both male and female fish was "adequate." In addition to considering the allocation of the catch in the primal problem, the dual problem considered the shadow prices or marginal value of the various sizes of fish, eggs, and cannery capacity, thus enabling the manager to view his decisions in light of the marginal values of these entities. As an example, the model was applied to a run of sockeye salmon in the Bristol Bay system. In the particular example, which was chosen to replicate the 1960 run, the additional value of the catch owing to optimality amounted to an ex-vessel value of a few hundred thousand dollars. In addition it appeared that the required processing time could be reduced by several days. The optimum allocation was obtained through conformance to the linear-programming model. The cost of this conformance was not, however, determined.

The Pacific salmon fisheries have been cited as an example of irrational conservation (Crutchfield and Pontecorvo, 1969). Much of this irrationality is reflected in the dissipation of a sizable fraction of the available economic rent, a situation which results from the open-access nature of the fishery and legislated inefficiency. The remedy for this situation is to alleviate the open-access and inefficiency problem. Such alleviation would require the dissolution of rather formidable institutional problems. In the present paper, we examine the salmon problem from a slightly different vantage point than Crutchfield and Pontecorvo. We examine the salmon problem under the status quo; we do not consider the optimal amount of gear or its efficiency (this should not, however, be construed as reflecting any diminution in the importance of these problems); rather we consider, as an interim approach, whether it is possible, under the stringent condition of knowing in advance the structure of the run, to increase the value of the fish on the dock by optimally allocating the catch among the days of the run.

The traditional approach to salmon management might be considered, at the risk of several simplifications, as consisting of (1) forecasting the magnitude of the run; (2) setting an escapement goal and a catch implied by the forecast and the escapement; and (3) daily fishing closures and other devices which allocate the catch in varying quantities to the days of the run. The traditional approach, then, also involves an allocation of the catch to the days of the run. In the traditional approach, the allocations are usually based on the experience of management biologists. Although the objectives of their allocations are not always clearly and explicitly stated, there is a tendency for the primary objective of management to be simply the attainment of the escapement goal. Our approach is to use the theory of linear programming to advise on a non-intuitive optimum allocation of the salmon catch among the days of the run where the objective of management does not explicitly involve escapement. Rather, we develop our allocation strategy to maximize the value of the catch on the dock given a variety of constraints which include the necessity for a given number of fish to escape the fishery. The objective of maximizing the value of the fish on the dock and the constraints explicitly define the objectives of the management scheme.

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We consider these problems in three additional sections. In the first, we describe the linear-programming allocation model, which we believe to be applicable, with simple modifications, to a variety of salmon management situations. In the second, we consider how the model might be applied to a run of salmon in the Naknek-Kvichak system of Bristol Bay, Alaska. As an example, we choose data from the 1960 run to that system and obtain an optimum allocation of large and small, male and female fish, on each day of the run to the daily catch. This optimum allocation served to maximize the value of the fish on the dock subject to constraints which ensured that the catch did not exceed the daily run, that the catch would be less than the cannery capacity, and that an "adequate" escapement, both in terms of the number of eggs and sex ratio, passed the fishery. Thus, in addition to managing the run by a non-intuitive optimum allocation and satisfying an escapement goal, we also considered the quality of the run in terms of its sex and age composition. In order, however, to achieve this optimum allocation we needed certain data on the structure of the run in advance and we also needed a mechanism by which we could select large and small male and female fish. It would most likely be impractical to have either a precise prediction of the daily run or an ability to select, with high precision, large or small, male or female fish. We show that even if we had the necessary data, a technique for precise selection of the various entities of fish, and maintained the 1960 escapement and sex-ratio conditions, optimum allocation would yield us a catch having a value of several hundred thousand dollars more than the actual catch. Thus given the cost of obtaining the necessary information to perform the optimum allocation and the constraints extant in 1960, it is questionable whether biological management could yield a better allocation than that which was obtained. This serves to re-emphasize the approach of Crutchfield and Pontecorvo, indicating that the system is most sensitive to variables which lie outside the objective and constraint equations specified in the present paper. On the other hand, our results show that it is possible, at least in terms of the model, to reduce the number of days during which the cannery operates and yet process the same number of fish. Furthermore as previously indicated, we constrained our example to fit the statistics of the 1960 run and thus we had, in our example, a nearly 1:1 sex ratio; but as we indicate later, we could have caught a considerably larger number of male fish and still would have had sufficient male fish in the escapement to ensure the efficient production of fertilized eggs. And finally the model was quite sensitive to decreasing the escapement but unfortunately there is little guidance in the literature which would indicate the optimum escapement for the Naknek-Kvichak system and furthermore there appears to be little hope of learning the magnitude, in the reasonably near future, of the optimum escapement for the Naknek-Kvichak system. Thus evaluation of the cannery processing time, catch problem, and relaxation of sex ratio and escapement constraints might result in an added value to the catch which would make some attempts at allocation practical. We also, in the second section, place some stress on interpretation of the shadow prices of the various variables in the problem. This is of interest to operations researchers because it provides an example, in addition to those conventionally used, of an application of the interpretation of the linear-programming primal-dual relation. The shadow prices are of interest to the fishery manager because from them it is possible to impute values to the various resources under the manager's control, and, in making a decision, the manager can thus consider these values which, as we show, are not always intuitively obvious. In the third and final section we conclude the paper with a general discussion of salmon management in a linear-programming setting.

MODEL

Most linear-programming models generally involve finding values X_i which maximize (or minimize) an objective function $\Sigma c_i X_i$, subject to a set of constraints each of which has the form $\Sigma \beta_i X_i \leq L_j$, where the inequality can be in either direction or can, in fact, be an equality.

The β_i's and the L_j's are constants appropriate to a particular problem. The details of the LP (linear-programming) procedure can be found in the many treatises on the subject (e.g., Gass, 1964) or in most texts on operations research (e.g., Hillier and Lieberman, 1967).

In our application of the LP model, we maximize the following objective function

$$Z = \sum_{i=1}^{M} \sum_{j=1}^{N} c_{ij} X_{ij}, \qquad (1)$$

where M refers to the total number of age-sex categories and N refers to the days of the run. The variable X_{ij} is the number of fish caught in the ith entity on the jth day of the run and c_{ij} corresponds to the value of the fish caught in the ith entity on the jth day (Table 1). The age-sex category classification results from the fact that salmon runs are comprised of a variety of age-groups. Because each age-group is usually of a different average size, the individuals in each age-group also have a different average value which we denote by c_{ij}. It should be mentioned that size is not the only criterion which can be used for classification. For example, in the Naknek-Kvichak run of Bristol Bay, the sex of the fish can also be used because within an age-group the male fish tend to be larger than the female fish and thus more valuable in terms of weight of fish-flesh; but, on the other hand, the eggs of the females are a valuable commodity and thus the per-pound value of females may be greater than the per-pound price of males. If the value of the fish were constant during the course of the run, we could replace the c_{ij} with c_i and the allocation problem would become rather uninteresting. But the value, however, does tend to vary during the course of the run. One reason for this is a deterioration of the quality of fish, as indicated by declining oil content and reduction in color intensity with the progression of the run. Another way in which c_{ij} could vary is that the average value of the fish on a particular day would tend to vary during the course of the run because of a within-entity trend in the average size of the fish during the course of the run; this, however, is not considered in the present paper. It is obvious that, if we had sufficient information, we could establish a large number of different c_{ij}'s.

TABLE 1.—Linear program model notation.

M	—	The total number of age-sex categories.
N	—	The total number of days in the run.
X_{ij}	—	The number of fish in the ith age-sex category which are caught on day j of the run.
c_{ij}	—	The value of a fish caught in the ith age-sex category on day j of the run.
R_{ij}	—	The number of fish in the ith age-sex category which run past the fishery on day j of the run.
K_j	—	The capacity, in numbers of fish, of the canneries on day j of the run.
K'	—	The total seasonal capacity of the canneries in numbers of fish.
W_{ij}	—	The number of fish of the ith age-sex category in the escapement on day j of the run
a_i	—	The average number of eggs in each fish of the ith age-sex category.
T	—	The total number of eggs contained in the escapement and catch.
E	—	The minimum number of eggs required in the escapement.
M	—	The total number of males in the escapement and catch.
F	—	The average fecundity of the female age-sex categories, expressed in number of eggs.
H	—	The sex ratio desired in the escapement, expressed as the number of females per male.
L_i	—	The number of fish of the ith age-sex category desired in the season's escapement.
S_i	—	The number of fish in the total season run of the ith age-sex category.
$P(j)$	—	The proportion of the run that arrives by day j of the run.
$P'(j)$	—	The proportion of the run that arrives on day j of the run.

As indicated previously, equation (1) is maximized subject to a variety of constraints. For the salmon problem, the first set of constraints is rather obvious and constrains the catch, of any entity, on any day, to be less than, or equal to, the number of fish in that entity in the run. These constraints are of the form

$$X_{ij} \leqslant R_{ij}. \qquad (2)$$

X_{ij} is always $\geqslant 0$ and R_{ij} is the number of fish of the ith entity which run past the fishery on the jth day. There can be as many as $M \times N$ constraints of this form, but in some applications, either the number of entities or the number of days will be collapsed owing to either the nature of the problem or a lack of information. Note also we can easily "close" the fishery for

any entity or all entities on any day simply by setting the appropriate $R_{ij} = 0$.

The second set of constraints constrains the catch on any day to be less than the daily capacity of the canneries. Thus, we have the set of constraints,

$$\sum_{i=1}^{M} X_{ij} \leq K_j \qquad (3)$$

where K_j is the capacity of the cannery or canneries on the jth day. Another form of this constraint might be incorporated in situations such as in the Bristol Bay fishery, which has a short season, is remote from supply points, and thus has a finite seasonal capacity; these constraints can be expressed by

$$\sum_{i=1}^{M} \sum_{j=1}^{N} X_{ij} \leq K. \qquad (4)$$

The constraint is redundant if $K \leq \sum_{j=1}^{N} K_j$.

and hence is only used when the season's capacity is less than the sum of the daily capacities, i.e.,

$$K \leq \sum_{j=1}^{N} K_j.$$

The next set of constraints results from the need to ensure that an adequate number of fish escape the fishery and are thus permitted to spawn. We formulate this constraint in terms of the egg complement of the number of females escaping the fishery rather than the number of females escaping per se or the total number of fish (males and females) escaping.

In order to formulate this constraint set, we define T as

$$\sum\sum a_i W_{ij} + \sum\sum a_i X_{ij} = T \qquad (5)$$

where a_i is the average number of eggs in each fish in the ith entity, W_{ij} is the escapement of fish in the ith entity on the jth day and thus T is the egg complement of the escapement and the catch. Now if we need a minimum number of eggs to represent the escapement, a quantity which we denote by E, we must have

$$\sum\sum a_i W_{ij} \geq E. \qquad (6)$$

So substitution of (6) into (5) yields the constraint set conformable to the X_{ij}'s of our other constraints, viz.

$$\sum\sum a_i X_{ij} \leq T\text{-}E. \qquad (7)$$

We can see that by using the same reasoning we could construct a constraint set which would constrain the egg complement to be less than some maximum egg complement.

Our next constraint involves the sex ratio of the spawning fish. The utility of allowing a particular egg complement to escape the fishery could be negated by not allowing a sufficient number of males to escape for the purpose of fertilizing the eggs. In order to ensure that an adequate number of males escape the fishery, we formulate the necessary constraint by noting that

$$\sum W_j + \sum X_j = M \qquad (8)$$

where in this particular equation the W's refer to the number of males in the escapement and the X's to the number of males in the catch and M to the total number of males in the run. Now to satisfy our constraint, we must have

$$\sum W_{ij} \leq \frac{E \times H}{F} \qquad (9)$$

where the sum extends over all male entities and days of the run; F is the average fecundity of the female entities in the run, and H is the desired male to female sex ratio. Substitution of (9) into (8) then yields the desired constraint

$$\sum X_{ij} \leq M - \frac{E \times H}{F} \qquad (10)$$

This constraint can also be formulated in a variety of ways.

In addition to escapement goals established in terms of eggs and a sufficient number of males to fertilize the eggs, we desired to establish, for some sample problems, escapement goals in terms of total numbers of fish of each entity in the escapement; this would greatly simplify the simulation of the actual escapements for any historic salmon season. Hence, we developed the constraint

$$\sum_j W_{ij} \geq L_i \tag{11}$$

where L_i is an escapement goal set for entity i which would ensure escapements identical with any historic year. To make (11) conform to our constraint set, we note that

$$\sum_j W_{ij} + \sum_j X_{ij} = S_i \tag{12}$$

where W_{ij} and X_{ij} are the escapement and catch (respectively) of entity i on day j and S_i is the total season's run of entity i, we can substitute (11) into (12) and get the constraint in the proper form:

$$\sum_j X_{ij} \leq S_i - L_i. \tag{13}$$

Thus a seasonal limit can be placed upon the catch of any entity i.

As indicated earlier, once the objective function and the constraints have been formulated, then optimization of the objective function, given the constraints, is a standard procedure outlined in some detail in the literature, and, with a computer facility, is a relatively simple task. There are, however, interpretations which are of further interest than the solution of the objective function. These interpretations rest in the primal-dual relationship of the LP problem.

In order to demonstrate this relation, we will denote a general form of the primal problem as

$$\text{maximize} \quad \sum_{i=1}^{m} c_i X_i$$

$$\text{subject to} \quad \sum_{i=1}^{m} a_{ij} X_i \leq b_j \qquad j = 1, \ldots, n \tag{14}$$

where we have used slightly different notation than in the salmon problem, but the analogue between (14) and the salmon problem should, nevertheless, be quite clear. If (14) is the primal, then the dual of this primal is

$$\text{minimize} \quad \sum_{j=1}^{m} b_j Y_j$$

$$\text{subject to} \quad \sum_{j=1}^{m} a_{ij} Y_j \geq c_i \qquad i = 1, \ldots, m \tag{15}$$

In the primal we are allocating scarce resources, the b_j's (number of fish in the run, cannery capacity, egg complement, and male fish), among the i activities (which consist in our problem of catching a particular entity of fish on a particular day). The intensity of the activity is X_i, the catch of the ith entity on the jth day and the "profit" per unit of the activity is, of course, c_j. It might be mentioned at this point that most LP computer codes provide as output the value of slack variables which in (14) is the difference between the right-hand side and the left-hand side of the constraint equations. The slack variables have a rather important interpretation for the salmon problem in that the slack variable in the run constraint (2) is the escapement; in the cannery constraint (3), it is the unused capacity of the cannery which we might want, in viewing the problem from a different context, to minimize; in the egg constraint (7), it is the number of eggs which are not caught that could be caught —another quantity which we might wish to minimize—and finally we have the slack variable associated with constraint (10) denoting the number of males which are not caught, but

could be caught, and which we might, similarly, wish to minimize. Thus, for example, we simultaneously derive, by virtue of the LP model, as we have formulated it, *both* the escapement and the catch.

Now, in the dual, we can place unit values on the scarce resources rather than on the levels of the activities, as in the primal. It is thus helpful, in making management decisions, to know the imputed unit value of a unit of cannery capacity, a large male fish, an egg, etc. These imputed values are commonly known as shadow prices and correspond to the optimal values of the Y_j's in (15); they will be discussed briefly in our interpretation of the salmon model. It should also be mentioned that we have slack variables in the dual formulation just as we had slack variables in the primal.

The dual slack variables can be viewed as opportunity costs in the sense that if we fail to meet a constraint, this is an opportunity foregone; and the dual slack variable then gives the value foregone by the "bad" management either of nature (that is, the vagaries induced in the system which are uncontrollable by the management agency) or of the management agency.

Finally, it is worth noting a feature of the shadow prices vis-á-vis the relation of the right- and left-hand side of the constraint equations. If in some solution of a particular problem, the right-hand side becomes equal to the left-hand side, then we say the constraint is binding. If the constraint is binding, then the shadow price has some positive value, namely the imputed value of an additional unit of scarce resource; but if, on the other hand, the constraint is not binding, then an additional unit of the resource is "free" within the bounds of the problem formulation—consequently the shadow price of the free resource is zero.

EXAMPLES BASED ON THE NAKNEK-KVICHAK RUN

As an example, we have decided to consider the implications of a LP approach to implementing the management framework of one of the most important salmon runs in North America, the sockeye salmon run to the Naknek-Kvichak system of Bristol Bay, Alaska. Our approach was to use the LP model described in the previous section employing actual data where available for the constraints and the objective function. Although we examined behavior of the model for several of the years for which we had data, we are presenting in this paper our partial analysis for the 1960 run only. Initially, we indicate how we assigned values to the various coefficients in the problem and then we give the actual examples.

First, we assigned values to the objective function (1) which is to be maximized. With respect to the number of entities in the objective function, there is a relatively large number of ocean-age groups represented in the Naknek-Kvichak run, but the very great majority are either the relatively large .3 ocean-age fish or the relatively small .2 ocean-age fish. Because, as we will see in subsequent paragraphs, the male fish are valuated differently than the female fish, we used four entities: male or female, .2 or .3 ocean-age fish.

Next, in order to assign values c_{ij} to each entity in the objective function according to the conventional per-fish management unit, we used the aforementioned observation that the male fish in each age group tend to be larger and hence more valuable than the female fish. On the other hand, the eggs which are contained in the females are processed into a caviar-type product, "sujiko," by Japanese firms in the Bristol Bay canneries. Thus, the females, because of the eggs which they contain, are more valuable than males of the same weight. Taking these factors into consideration and using an average ex-vessel value of $0.25 pound, we have computed the average value for each entity. These calculations are set forth in Table 2 which shows, among other things, that the added value of eggs tends to offset the reduced value of females relative to males of the same age class.

As indicated previously, the $0.25 pound is an *average* value and it should be emphasized at this point that it is not a computed average since generally speaking a fixed price is paid for fish throughout the season. But as we indicated earlier, some fish are certainly more

TABLE 2.—Computations used to determine the value of each entity classification for the model.

Entity	Sex	Age	Average weight[1]	Average no. of eggs[2]	Average value c_i
			Lb.	*Number*	*$*
1	Male	2-ocean	5.1	--	1.28
2	Male	3-ocean	7.4	--	1.85
3	Female	2-ocean	4.5	3,700	1.38
4	Female	3-ocean	6.2	4,384	1.84

[1] With the price of salmon at $0.25/pound, the value of each entity can be calculated as follows:

entity 1 — 5.1 X $0.25 = $1.28;
entity 2 — 7.4 X $0.25 = $1.85;
entity 3 — 4.5 X $0.25 = $1.13;
entity 4 — 6.2 X $0.25 = $1.55.

[2] With the price of salmon eggs at $0.50/pound, the additional value of each female entity can be calculated as follows (it should be noted that eggs were not processed in 1960):

$$\text{entity 3} - \frac{3{,}700 \text{ eggs} \times .061 \text{ g/egg}}{453.6 \text{ g/lb}} \times \$0.50 = \$0.25$$

$$\text{entity 4} - \frac{4{,}384 \text{ eggs} \times .061 \text{ g/egg}}{453.6 \text{ g/lb}} \times \$0.50 = \$0.29$$

valuable than others. Thus, we deduced that this fixed price must reflect an average value and we note, parenthetically, the important point that the bias and precision (we take the liberty of using these terms in the statistical sense even though the estimation procedure may not be statistical in nature) with which this average is estimated is a subject of significance to the management of the salmon stocks.

In addition to the value of salmon differing among entities, the value of salmon usually deteriorates within an entity during a season. Thus, even though a fixed price is paid for salmon during a season, the value decreases owing to a reduction in quality. For example, the value of pink salmon may be 25 % less near the end of the run than near the beginning of the run. The decline in value of red salmon is not so severe, amounting to a range of about $0.03/pound from the beginning to the end of the season. (While a few cents decline in value during the course of the season may seem to be a negligible quantity, we must remember that this factor must be multiplied by the several pounds in weight of each fish and the several million fish that are involved in the value reduction.) So just as we deduced $0.25/pound to be an average price among entities, we must likewise deduce that the values tabulated in Table 2 are average values for each entity for the season. In the Naknek-Kvichak run, which usually begins around June 27 and ends around July 15, the decline in value appears to be centered on July 4.

In order to arrive at a unique allocation, we must deduce how the c_{ij}'s for each of the j days of the run differ from the average of the c_{ij}'s listed in Table 2. The ideal way of doing this would be to develop a model which is descriptive of the value change during the run. Unfortunately, we have no information upon which to base such a model, so we used three arbitrarily chosen functions to describe the day-to-day value change of the salmon. An example of these is shown in Figure 1.

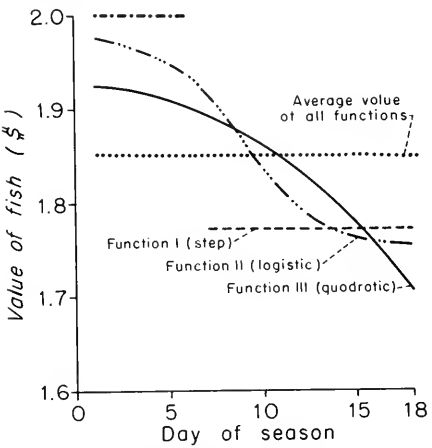

FIGURE 1.—Value functions as objective function coefficients in the linear-programming model for entity 2 (large male fishes).

Next we needed to determine the quantity, in the objective function, of N, the number of days of the run. We utilized an empirical equation presented by Royce (1965) to obtain both N and the daily run for each entity R_{ij}. The function Royce used for each of several years to describe the temporal change in the Naknek-Kvichak catch is

$$P_k(j) = \frac{1}{1 + e^{-(a_k + b_k j)}} \qquad (16)$$

where k specifies the year of the run, a_k and b_k are constants, j is time in days, and $P_k(j)$ is the cumulative proportion of the total catch. In order to choose N, we defined the fishing season to be those days for which $.05 \leq P(j) \leq .95$ and the difference between the initial and closing day was, to the nearest integral value, set equal to N.

We also used expression (16) to obtain R_{ij} from $P_k(j) = P_k(j) - P_k(j-1)$ and then $R_{ij} = P_k(j)\ S_i$, where S_i is the number of fish of the ith entity in the run during the year under consideration. The assumptions involved in obtaining the R_{ij}'s are (1) the catch is proportional to the "average" run as defined by the fitted curve (16); and (2) R_{ij} is proportional to $R_{.j}$. It should also be mentioned that our R_{ij}'s are certainly different from the traditionally used R_{ij}'s because the latter are based on counts made several days after the fish enter the fishery area. This, however, is not important in the allocation whereas the relative daily size of the run is important. We feel that these assumptions are reasonable for the present model until more accurate information can be obtained on the behavior of the fish in the run.

On some occasions, the catch in Bristol Bay is limited by the cannery capacity. This capacity can be, of course, adjusted by the industry, but it appears that, according to Mathews (1966), 1 million fish per day is a maximum capacity and we used this value in constraint equation (2). It would appear that the most important assumption implicit in the nature of this constraint is independence among the days; that is to say, we assume that processing a certain number of fish on one day does not affect the processing capacity on the next day. A second assumption is that the maximum capacity is not dependent upon the average size of the fish processed. There is some question, when the cannery operates near peak capacity, as to the effect of overtime payments to cannery employees and to the effect of processing large numbers of fish on the quality of the pack.

For our example, of the 1960 run, the total season constraint was not reached and so this constraint had no effect on any of the examples which we present. It is relevant to note, however, that if this constraint is needed, then the maximum number of fish ever processed (up until 1969) in the Naknek-Kvichak system was 19.1 million.

The next constraint is the escapement constraint. Despite the fact that the level of escapement has, for salmon stocks, been the primary management criterion, there is very little documentation on the proper escapement level for many systems. The latest synthesis of the extensive work on the Naknek-Kvichak system is addressed to the problem of optimum escapement in that particular system as well as others (Burgner, DiCostanzo, Ellis, Harry, Hartman, Kerns, Mathisen, and Royce, 1969), but unfortunately no advice on optimum escapement is given for the Naknek-Kvichak. Another study implies, on the basis of limited data, that escapements beyond about 10 million fish will not result in any increased productivity of smolts (Mathisen, 1969: Fig. 6), and thus if we make a simplifying assumption and assume that marine survival rates are independent of density, we can further assume that an increased return will not accrue from an escapement larger than 10 million fish (very roughly 20 billion eggs).

Despite the fact that we do not "know" the optimal minimal escapement for the Naknek-Kvichak system, it is clear that there must be some level which is, in some sense, optimal. This statement must, of course, be tempered with our knowledge of the cyclical nature of the run.

Because of our uncertainty, we examined the model under a variety of escapement conditions but keeping under each set, what would appear to be, according to studies by Mathisen (1962), a conservatively high male-to-female sex ratio of at least one male for every three females. We found in general, as one might expect, that as we increased escapement we decreased the value of the catch. The objective function was quite sensitive to this manipulation, focusing again upon the need for a well-defined escapement policy. What is not so obvious, however, as we will see subsequently, is that as we change the escapement level, we can obtain considerable changes in the imputed values of the var-

ious entities. The 1960 run had an escapement of 31 billion eggs. We estimate that this escapement was partitioned among the entities as follows:

Entity 1, 57.5 % or 8,136,000 fish escaping
Entity 2, 43.4 % or 210,000 fish escaping
Entity 3, 74.8 % or 7,964,000 fish escaping
Entity 4, 30.8% or 389,000 fish escaping

It is interesting to note that in addition to 8,350,000 females which were estimated to have escaped in 1960, there were, in addition, 8,300,000 males that escaped, signifying a nearly 1:1 sex ratio.

Our general approach in presenting our particular 1960 run example was to concentrate upon simulating the actual events in 1960 by using the above run data in equations (13) and (7), the fecundities listed in Table 2, and the logistic value function. We also present some results where we effectively drop equation (13) and replace it with equation (10) using a low escapement of 5 billion eggs in order to demonstrate the sensitivity of the model to various escapement goals.

In Figures 2, 3, 4, and 5, we depict the optimum allocation of the catch which we obtained using the actual 1960 escapement data. The figures also include the smoothed catches derived from Royce's work. From these figures,

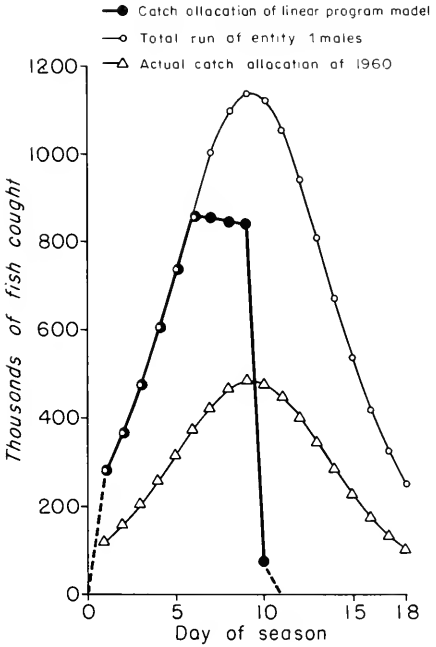

FIGURE 2.—Year 1960 small male catch allocations (entity 1). The actual catch allocations are the smoothed average values obtained from Royce (1965).

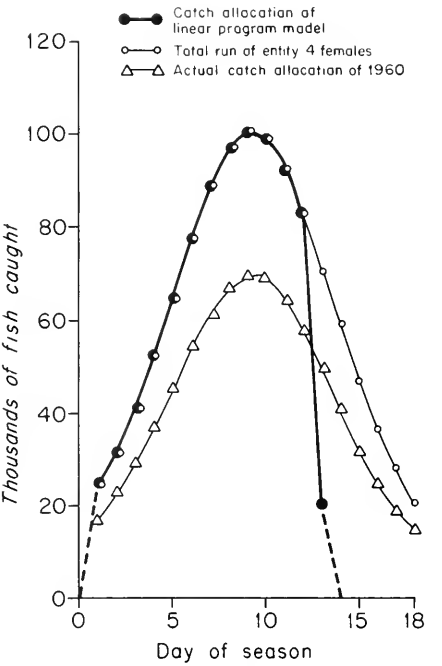

FIGURE 3.—Year 1960 large female catch allocations (entity 4). The actual allocations are the smoothed average values obtained from Royce (1965).

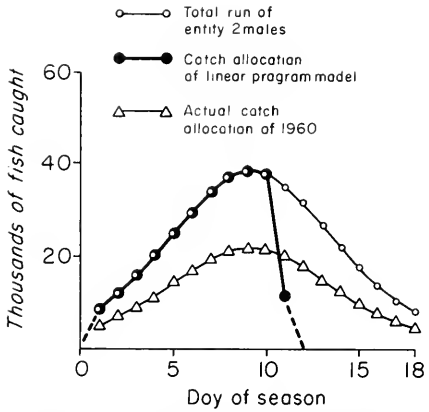

FIGURE 4.—Year 1960 large male catch allocations (entity 3). The actual catch allocations are the smoothed average values obtained from Royce (1965).

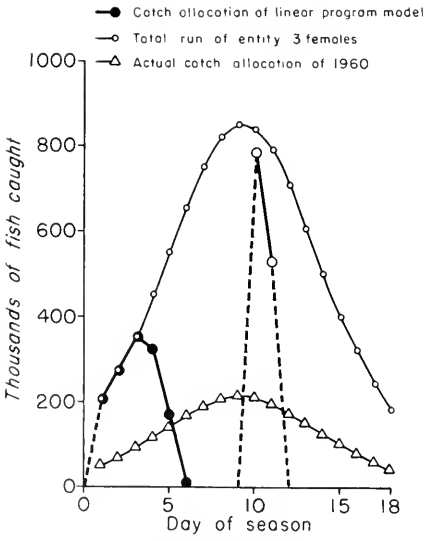

FIGURE 5.—Year 1960 small female catch allocations (entity 3). The actual catch allocations are the smoothed average values obtained from Royce (1965).

we can observe various properties of the optimally allocated catches. For example, in Figures 3 and 4, the catch allocation of the early season is identical to the number of fish in the run. These large male and large female fish are more valuable than the smaller fish and most valuable in the early part of the season because of the value function. Hence, all the large fish available are caught until the season's limit for each of the large-fish entities has been reached. We must be cautious here, however, because as we point out in our discussion, there is a possibility of modifying the characteristic of the run by selective fishing.

In order to interpret Figures 5 and 6, we must realize that the next most valuable fish are the small females. The difference in price between small females and small males increases through the season. This owes to the fact that small females weigh less than small males and thus decrease in value less towards the end of the season. (Recall that the small females are more valuable than small males only because of the eggs which they contain.) We have assumed that the value of the eggs is constant throughout the season.

The optimal solution shows that the cannery is at capacity from days 4 to 10 with as many small males being caught through day 6 as are available, and as many additional small females being caught as the cannery can process. On days 7 to 9 there are enough fish exclusive of the small females to bring the cannery to capacity. However, by day 10, the season's limit of small males has been caught and small females must be caught to keep the cannery at capacity. It must be remembered that the price difference between females and males is greater later in the season. For example, if a small female is caught instead of a small male on the 6th day of the season, the increased profit is \$1.430 — \$1.337 = \$0.093, where the figures are the value of small females and small males, respectively, on day 6 for the logistic value function which we used. But if a female is caught instead of a male on day 10, the increased profit is \$1.371 — \$1.270 = \$0.101, for the same value function. Hence, while the cannery is being operated to capacity and since the total num-

ber of fish of any entity is fixed by the seasonal limit, it is more profitable to catch the small, less valuable, males earlier in the season, which may seem contrary to the intuition. The implications of the optimal allocation are considered in the discussion and conclusions section.

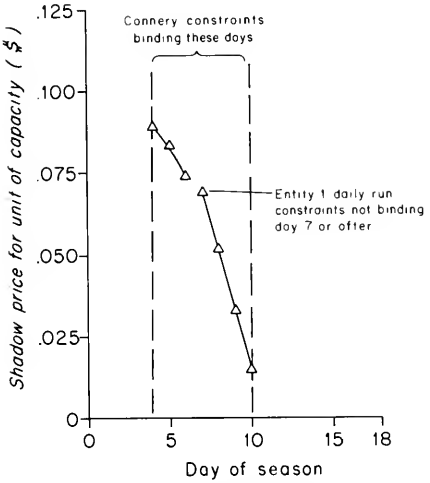

FIGURE 6.—Shadow prices for daily cannery constraints —an example in which seasonal entity limits are imposed.

As indicated previously, the shadow prices are useful in considering various management implications. We consider, as examples, the egg shadow prices, the cannery shadow prices, and the run shadow prices. It might be mentioned somewhat parenthetically that although the shadow prices can be explained and interpreted as in the following paragraphs, in the LP calculation, they are not found in this manner. The shadow prices are calculated as simultaneous results of an iterative solution procedure and include the results of previous iterations. In fact, the shadow prices associated with each constraint at the end of each iteration are used to determine how to manipulate the matrix to improve the objective function in the subsequent iteration. With very involved problems, it might not be possible to examine the shadow prices as below, and in any case, only a good deal of insight into the problem permits their delineation in this manner. One other caution is that while applying the following equations to determine a total increase in profit, care must be taken to see that the same constraints remain binding or nonbinding. Once a constraint changes from binding to nonbinding, the solution basis changes, also changing the relationships between variables and constraints.

The increase in value of the objective function corresponding to a relaxed egg constraint (allowing one more egg in the catch) is the shadow price associated with the egg constraint. The shadow price is dependent upon which, if any, of the constraints in the LP model are binding. If the egg catch constraint[4] is not binding, indicating that the value of this catch scheme is not being limited by this constraint, then the shadow price associated with the egg constraint is zero. If the egg catch constraint is binding, shadow prices associated with the egg constraint, depending upon which of the other constraints are effective, can be calculated.

The imputed value of an egg, its shadow price, thus depends on whether the cannery constraint is binding. Now, if the cannery constraint is binding on day j (indicating that the maximum number of fish are being processed and the addition of a single or marginal fish to the processed catch requires that a fish already existing in the catch must, to maintain the constraint, be replaced by the marginal fish), and the entity 4 run constraints are binding throughout the season (indicating that all large females are caught), then the shadow price associated with the egg catch (ESP) constraint is

$$ESP = (c_{3,j} - c_{1,j})/3{,}700$$

where day j is a day on which the entity 3 run

[4] Although the egg constraint arose from a minimum egg escapement requirement, it was necessary to convert it to a maximum egg catch requirement constraint for use with the LP model, as was described earlier. It is convenient to think of the constraints in terms of "egg catch" for purposes of discussing the shadow prices.

constraint is not binding, i.e., there are entity 3 females available to be caught. The formula indicates that allowing the catch of one more egg essentially allows the catch of 1/3,700 of an entity 3 female which requires the escapement of 1/3,700 of some other fish since the cannery constraint is binding. The fish to be included in the escapement is, of course, the low-valued entity 1 male.

If the cannery constraint on day j is binding, but there is a day when the entity 4 run constraint is not binding, and since the value of a large female per egg is greater than the value of a small female per egg, the value of allowing the catch of an additional egg is

$$ESP = (c_{4,j} - c_{1,j})/4{,}384$$

where day j is a day on which the entity 4 run constraint is not binding.

If the cannery constraint is not binding on day j then catching additional females does not require the escapement of an equal number of males, or, in other words, the addition of the marginal fish does not require the release of a fish extant in the catch. If, however, all the entity 4 run constraints are binding through the season, then

$$ESP = c_{3,j}/3{,}700$$

where day j is a day on which the entity 3 run constraint is not binding. Or, if an entity 4 run constraint is not binding on day j and again since the value of a large female is greater than that of a small female,

$$ESP = c_{4,j}/4{,}384$$

The next shadow price that we will evaluate is the increase in value of the ability to process an additional or marginal fish. The imputed value of processing a marginal fish is called the shadow price of the cannery constraint (CSP), and we must remember that this marginal fish only has an imputed positive value if the cannery constraint is binding. In other words, we can impute a value to an additional unit of cannery capacity. Following the format above, the shadow prices for the cannery constraint can be outlined. For emphasis, we repeat again that if the cannery constraint is not binding, indicating that the value of this catch scheme is not being limited by this constraint, then the shadow price associated with the cannery constraint is zero. If the cannery constraint is binding, shadow prices associated with the cannery constraint, given which other constraints are effective, can be determined.

The objective function will be increased by an amount equal to the value of the additional fish which is included in the new catch scheme (the scheme arising from relaxing the cannery constraint), and hence the most valuable fish will be caught. If run constraints are not binding, the shadow price is

$$CSP_j = c_{2,j}$$

since the large males are the most valuable. As the run constraints become binding for the more valuable entities, the shadow price of the cannery constraints is equal to the value of the most valuable entity available.

If, for example, the egg catch constraint is binding as well as the entity 2 run constraint, the shadow price of the cannery constraint is

$$CSP_j = c_{4,j} - \left(\frac{4{,}384}{3{,}700}\, c_{3,j}\right).$$

The modification of the equation assures that enough eggs will escape (via entity 3 female) to enable the catch of the more valuable entity 4 female.

To emphasize the effect of reduced escapements and concomitant binding egg catch constraints, we employed the 1960 run model but dropped equation (13) and used an effective escapement of 5 billion eggs in equation (10). If the egg catch constraint is binding as well as entity 4 run constraints, the only fish available for catching are the entity 1 (small males), since no more females can be caught without violating the egg catch constraint. Hence

$$CSP_j = c_{1,j}.$$

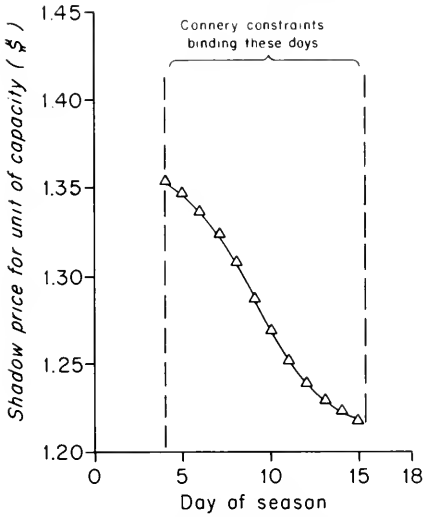

FIGURE 7.—Shadow prices for daily cannery constraints —an example in which seasonal entity limits are not imposed.

As an example of this situation where we have an escapement of 5 billion eggs, consider Figure 7, where the curve representing the cannery shadow prices is exactly the value function curve for entity 1 males. If, however, we contrast the 5 billion egg constraint situation with the actual 1960 run where the seasonal limit constraints are binding for all entities, then the improvement in the objective function attributed to the one unit of increased cannery capacity will only be equal to

$$CSP_j = c_{ij} - c_{ij^*}$$

since to catch a fish of entity i on day j another fish of entity i must be released on day j^* ($j^* \neq j$) to avoid breaking the seasonal limit constraint. Figure 6 gives an example of this situation. The particular example is taken from the LP model using the logistic value function and actual escapements of 1960 by entity (thus the seasonal limit constraints). In this case, the total number of fish processed remains the same (since season limits are binding for all entities already), but processing a fish earlier in the season can result in an increased profit because of the shape of the value-function curve.

The implications of Figure 6 are quite subtle. In Figure 6, until day 4, the cannery is not at capacity, and hence there is obviously no value associated with an increased unit of capacity. All fish are included in the catch allocation in the early-season, high-value situation. On day 4 the cannery is at capacity and some fish must be included in the escapement (excluded from the catch). Intuitively, we would expect the highest value fish to be caught. This is partly reflected by the continued catch of all entities 2 and 4 fish (the large males and females), but, keeping in mind the fact that catches in all entities are being limited by the seasonal limit constraints and the idea of the decreasing price differential between entity 3 and entity 1 fish, entity 3 fish become part of the escapement. This says that on days 4 to 6, an increase in the capacity of the cannery will result in an entity 3 fish being caught on that day and a less valuable entity 3 fish released later in the season (to avoid breaking the season's limit constraint). We can see in Figure 5 that the last day on which an entity 3 fish can be released is day 11. The value of an entity 3 fish on day 4 is \$1.445, the value of an entity 3 fish on day 11 is \$1.356. Hence we would expect to gain exactly \$0.089 by increasing the cannery capacity by one unit on day 4. Checking Figure 6, we see this is exactly what the graph shows for day 4. Days 5 and 6 can be calculated similarly.

On days 7 to 10, entity 1 daily run constraints are no longer binding, and hence an additional unit of cannery capacity could result in an entity 1 fish being caught on day 7. From Figure 5 the least valuable entity 1 fish in the catch scheme is on day 10, and one of these would go into the escapement. The increased value is

$$c_{1,7} - c_{1,10}$$

or

$$\$1.324 - \$1.270$$

or a profit increase of \$0.054. In Figure 6, the

shadow price of the cannery constraint on day 7 is shown as \$0.069. Note, however, that when an entity 1 fish is released on day 10 that the cannery is no longer at capacity on day 10, hence an entity 3 fish could be caught on day 10 if an entity 3 fish was released on day 11. This is an additional contribution to the shadow price for day 7 of

$$c_{3,10} - c_{3,11}$$

or

$$\$1.371 - \$1.356$$

or a profit increase of \$0.015. Adding this to the value of catching an earlier entity 1 fish gives \$0.054 + \$0.015 = \$0.069, and this is exactly what is shown for day 7 in Figure 6. Values for days 8 to 10 can be calculated similarly.

Next let us consider the run shadow prices (*RSP*). The increase in value of the objective function corresponding to relaxed run constraints allowing one more fish of any entity in the catch is the shadow price of that run constraint. If the cannery constraint is not binding, if seasonal run constraints are not used or are not binding, and if egg catch or male escapement constraints are not binding, there is no restriction other than the run constraint limiting the catch. Since there is a run constraint for each entity and day

$$RSP_{ij} = c_{ij}.$$

However, if the cannery constraint is effective on day j

$$RSP_{ij} = c_{ij} - c_{i^*j}$$

where entity i^* ($i^* \neq i$) is the fish that must escape to allow the catch of entity i since the cannery is already processing as many fish as possible. And if the egg catch constraint and or the male escapement constraint is binding, appropriate numbers of fish must be released when additional fish are caught. As an example of the behavior of the system when the 5 billion egg constraint is used, consider Figure 8. As shown in Figure 8, daily cannery constraints are binding from days 4 to 15, and, in addition,

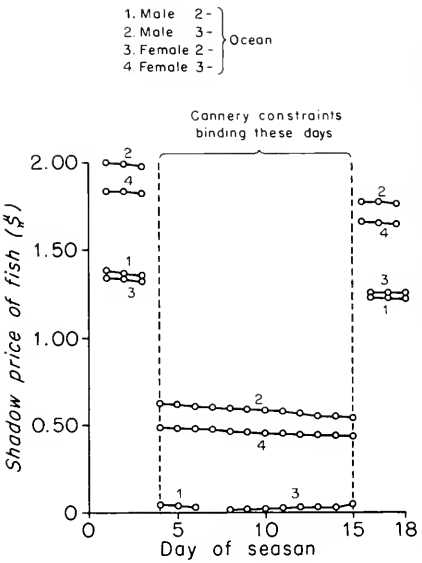

FIGURE 8.—Shadow prices for daily entity run constraints—an example in which seasonal entity limits are not imposed.

the egg catch constraint is binding. Although it is not illustrated in the figure, it is essential, in understanding the problem, to realize that the entire escapement satisfying the egg escapement constraint is made up of small (entity 3) females, and that the entire escapement satisfying the male escapement constraint is made up of small (entity 1) males. Also, all fish of all entities are in the catch scheme on days 1 to 3 and 16 to 18 (all days on which the cannery constraints are not binding).

In Figure 8, on days 1 to 3, the cannery constraints are not binding; and since entity 2 escapement is not being used to satisfy any constraints of any form, the run shadow prices on days 1 to 3 will be equal to the value function (this can be checked with the values listed in Appendix Table 2). In addition, since the male catch constraint is not binding, the run shadow price for entity 1 is also equal to its value func-

tion for the first 3 days of the season. This is not true for entity 3 or 4 since the egg-catch constraint is binding, indicating that no more eggs can be "caught" without breaking the constraint. Hence, when an entity 4 female is caught on a day 1 to 3 (catching 4,384 eggs), 4,384 eggs must be released on some other day of the season. This can be done with the smallest loss by releasing 4,384/3,700 entity 3 females (entity 3 females have 3,700 eggs). To further decrease the loss, the above fractional parts of an entity 3 should be released on a day on which there are entity 1 or 2 available to be caught, since this will keep the cannery constraint intact and not violate the male catch constraint since it is not binding. Remembering that the price differential between entity 1 and entity 3 increases over the season, it is desirable to catch the additional entity 1 fish as early as possible, which is on day 7. Then (note that all of the larger males are already in the catch scheme):

$$RSP_{4,1} = c_{4,1} - \frac{a_4}{a_3} c_{3,7} + \frac{a_4}{a_3} c_{1,7}$$

$$= \$1.941 - \frac{4,384}{3,700} \ (\$1.419) + \frac{4,384}{3,700} (\$1.324)$$

$$= \$1.828,$$

which can be seen on the graph as the shadow price for entity 4 day 1. To calculate the value of the run shadow price for entity 3 in days 1 to 3, we must realize that catching an entity 3 female requires the release of a female some other time during the season in order to avoid breaking the egg catch constraint. Since the entity 3 females are of lesser value than the fractional part of an entity 4 female which must be released to account for the 3,700 extra eggs caught, the release of this fish on a day on which there are entity 1 fish available will lessen the loss. As before, the price differential between entities 1 and 3 is smaller early in the season, and the earliest date on which an entity 1 male is available is day 7. For example,

$$RSP_{3,1} = c_{3,1} - c_{3,7} + c_{1,7}$$

$$= \$1.453 - \$1.419 + \$1.324$$

$$= \$1.358,$$

which is the value shown in Figure 8.

The run shadow price for entity 1 day 4 results from the catch of entity 1 on day 4 requiring the release of entity 3 (to avoid breaking the cannery constraint) which, in turn, allows the catch of an entity 3 and the release of an entity 1 on day 11 (the last day on which there are entity 3's *not* in the catch scheme). Essentially, what we have done is to exchange the catch of entity 1 and entity 3 for a time when the price differential is more favorable.

$$RSP_{1,4} = c_{1,4} - c_{3,4} + c_{3,7} - c_{1,7}$$

$$= \$1.353 - \$1.445 + \$1.419 - \$1.324$$

$$= \$0.003,$$

which is the value shown in Figure 8. Run shadow prices for entity 1 days 5 and 6 can be figured similarly. For entity 1 days 7 to 15, the daily run constraints are not binding, and hence, the run shadow prices are zero.

Run shadow prices for entity 2 days 4 to 15 (still referring to Figure 8) can be calculated as the difference between the value of an entity 2 and entity 1 on those days. The entity 1 must be released in order to satisfy the cannery constraints for those days. In addition, for days 4 to 6, calculating the value of this new availability of an entity 1 released in order to catch an entity 2 is essentially the same situation as calculating the shadow price of entity 1 on those days, and hence, the value of the scheme and the run shadow price is increased by that additional amount.

Run shadow prices for entity 3 days 4 to 7 in Figure 8 are zero since the run constraints of entity 3 for those days are not binding. For entity 3 days 8 to 15, they can be calculated as follows: If another entity 3 is caught on day 8, an entity 1 must be released on day 8 to maintain the cannery constraints. The catch requires the escape of an entity 3 fish on another day to maintain the egg catch constraint. In turn, if

the release of the entity 3 is on a day which a male entity is available, that entity can be caught without breaking the cannery constraint, e.g.,

$$RSP_{3,8} = c_{3,8} - c_{1,8} - c_{3,7} + c_{1,7}.$$

(Note that day 7 is the *first* day on which there is a male entity not already in the catch scheme, and hence, the price differential is smallest.)

$$RSP_{3,8} = \$1.404 - \$1.307 - \$1.419 + \$1.324$$
$$= \$0.002,$$

which is that value shown in Figure 8.

Run shadow price for entity 4 days 4 to 15 can be calculated by releasing and catching the fractional parts 4,384/3,700 of entities 3 and 1 as required to maintain cannery and egg catch constraints, in a manner similar to that for days 1 to 3. The run shadow prices for the male entities for days 16 to 18 (those days after the cannery constraints are no longer binding) are simply equal to the value of those entity-days, since they are not involved in satisfying any constraints of any form. The run shadow prices for the female entities on these days are a little more difficult to arrive at, since as they are caught, an equal number of eggs must be released, resulting in a slack cannery constraint which can be filled by catching additional male entities when available.

Consider, in contrast, Figure 9, where the run shadow prices are shown for a case in which we used the actual escapement for the 1960 run in constraint equation (13). The seasonal limit constraints are binding for all entities in this example. It follows that in every case when calculating a run shadow price for an entity day, the inclusion of an additional unit of any entity on that day implies that a unit of that entity must escape on some other day of the season to maintain the equality of the seasonal limit constraint for that entity.

In Figure 9, shadow prices are plotted for the daily run constraints for the particular example in which seasonal limits were imposed to achieve the actual escapements of 1960. Neither the egg catch constraint nor the male catch constraint was binding; the seasonal limit constraint was binding for each entity. Cannery constraints were binding from days 4 to 10, as indicated on the figure. In calculating the run shadow price for any entity on any day, note that whenever an additional fish is included in the catch scheme, a fish of that same entity must be eliminated from the catch scheme on some other day in order to maintain a valid seasonal limit constraint for that entity. Thus, for entity 1 days 1 to 3, the run shadow price can be calculated as the value of a fish included on the day being considered minus the value of the lowest valued fish of entity 1 in the catch scheme which must be released to keep the constraints

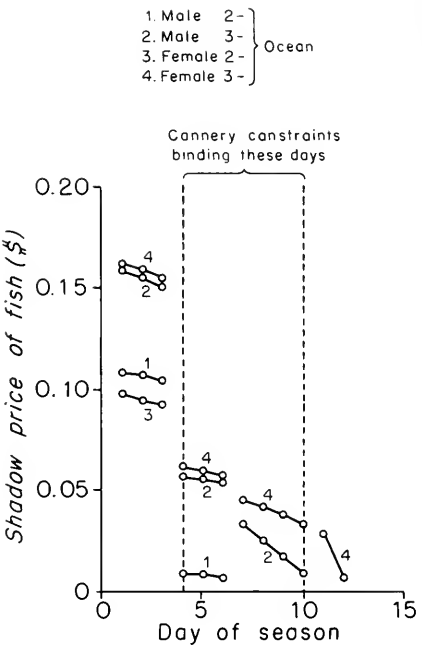

FIGURE 9.—Shadow prices for daily entity run constraints—an example in which seasonal entity limits are imposed.

effective. If this low-valued fish is released on a day on which the cannery constraint was binding, then the cannery constraint is no longer binding and a fish of another entity can be caught on that day if a fish of that other entity is available. For example,

$$\begin{aligned} RSP_{1,1} &= c_{1,1} - c_{1,10} + c_{3,10} - c_{3,11} \\ &= \$1.362 - \$1.270 + \$1.371 - \$1.356 \\ &= \$0.107, \end{aligned}$$

which is the value shown in Figure 9. The run shadow prices for entities 2 to 4 on days 1 to 3 are simply calculated as the value of the entity on that day minus the value of that entity on the lowest priced, and hence, last day on which it is included in the catch scheme. For example,

$$\begin{aligned} RSP_{3,1} &= c_{3,1} - c_{3,11} \\ &= \$1.453 - \$1.356 \\ &= \$0.097. \end{aligned}$$

Checking Figure 5, we see that day 11 was the last day on which entity 3 fish were caught, and Figure 9 shows that the value ($0.097) is correct.

Run constraints for entity 3 are not binding after day 3, so run shadow prices associative are zero.

To obtain the run shadow price for entity 1 for days 4 to 6, one may, for example, subtract from the value of entity 1 day 4, the value of entity 3 day 4 (which must be released to maintain the daily cannery constraints), subtract the value of entity 1 day 10 (which must be released to maintain the season entity 1 limit), and add the value of entity 3 day 10 (which can be caught since an entity 1 has been released on day 10); or

$$\begin{aligned} RSP_{1,4\text{-}6} &= c_{1,4} - c_{3,4} - c_{1,10} + c_{3,10} \\ &= \$1.353 - \$1.445 - \$1.270 + \$1.371 \\ &= \$0.009, \end{aligned}$$

which can be seen in Figure 9.

Run shadow prices for entities 2 and 4 for days 4 to 6 are similar. For illustration, entity 4 day 4 will be calculated:

$$RSP_{4,4} = c_{4,4} - c_{3,4} - c_{4,13} + c_{3,11}.$$

That is, the run shadow price of entity 4 day 4 equals the value of entity 4 day 4 less the value of entity 3 day 4 (to preserve the daily cannery constraint) less the value of entity 4 day 13 (to preserve the seasonal entity 4 limit) plus the value of entity 3 day 11 (since an entity 3 day 4 was excluded, this will be within the entity 3 seasonal limit constraint). Thus

$$\begin{aligned} RSP_{4,4} &= \$1.930 - \$1.445 - \$1.780 + \$1.356 \\ &= \$0.061. \end{aligned}$$

This is the value shown in Figure 9.

The run shadow prices for entity 1 after day 7 are zero since the daily run constraints are no longer binding. For entities 2 and 4 days 7 to 10, the run shadow prices can be calculated similarly. For example,

$$RSP_{2,10} = c_{2,10} - c_{3,10} - c_{2,11} + c_{3,11}.$$

That is, the run shadow price of entity 2 day 10 equals the value of entity 2 day 10 less the value of entity 3 day 10 (to preserve daily cannery constraints) less the value of entity 2 day 11 (the last day on which entity 2's are caught and, hence, the cheapest entity 2 which can be released to preserve the seasonal limit) plus the value of entity 3 day 11 (since an entity 3 was released on day 10, this will not fracture the seasonal entity 3 limit constraint). Thus

$$\begin{aligned} RSP_{2,10} &= \$1.836 - \$1.371 - \$1.812 + \$1.356 \\ &= \$0.009, \end{aligned}$$

which is shown as the correct value in Figure 9.

The run shadow prices for entity 4 on days 11 and 12 are easily calculated since the cannery constraints are no longer binding and this is the only entity being caught after day 12. Hence,

$$\begin{aligned} RSP_{4,11} &= c_{4,11} - c_{4,13} \\ &= \$1.808 - \$1.780 \\ &= \$0.028. \end{aligned}$$

It is interesting to note that the run shadow prices of entity 4 throughout the season are higher than those of entity 2 (see Figure 9), even though the value for an entity 4 is less, for any given day, than the value of entity 2. This is due to the optimal catch scheme which has catches of entity 4 until day 13, while entity 2 catches are only made until day 11. The effect is that when an additional fish of an entity is caught early in the season (requiring the release of a fish of the same entity later in the season), the entity 2 fish released is from day 11 of value \$1.812, the entity 4 fish released is from day 13 of value \$1.780. The value of the entity scheme then, decreases least (proportionately) for releasing the entity 4 fish.

DISCUSSION AND CONCLUSIONS

It can be seen that the linear-programming (LP) approach to salmon management, as with all other modelling approaches, involves a variety of assumptions which are either intrinsic to the procedure or to the way the procedure is applied to real-world problems. We have gone into some detail to show the richness of interpretations that the salmon model affords, and we believe that the application of this procedure to salmon management will provide increased guidance to and widen the spectrum of possible management decisions. The procedure we have used for the Naknek-Kvichak run is widely applicable to a variety of situations both within the Naknek-Kvichak sockeye salmon setting and to other salmon runs as well. The setting, the necessary data, and the formulation of the problem really depend on the problem situation. Our purpose was to demonstrate a conceptual method and we have chosen our data and examples accordingly.

There will naturally be differences of opinion in the formulation of the model (that is, different ways of expressing the constraint equations, some of which are indicated) or the appropriate data which should be used for actual management situations. These differences can at times be easily resolved by examining the sensitivity of the model to various data or formulation modifications.

Nevertheless, we should not ignore the assumptions which are implicit in the LP procedure. These are outlined by, for example, Hillier and Lieberman (1967), and constitute three concepts that should be recognized. These involve the linearity property of the model, the problem of divisibility, and the deterministic nature of the LP approach. In addition, it is important to consider that the LP approach models only static situations. First, the linearity property asserts, for example, that the value of any term in the constraint or objective function must be directly proportional to the level of activity involved. Expressed in another way, the relation between the level of an entity and its contribution toward filling the constraint or modifying the objective function must be a straight line passing through the origin, a condition not often met in practice but frequently approximated. Furthermore, there should be no synergism among the terms of the objective or constraint functions. For example, the unit catch value on day j for the ith entity, c_{ij}, cannot be affected, a posteriori, by the unit catch value on day j-1 for the ith entity $c_{i,\ j\text{-}1}$. The problem of divisibility refers to the fact that the LP approach that we have used gives solution values which are not necessarily integers. A usual practice is to round solutions to the nearest integer value, thus avoiding the embarrassment of having, e.g., 7,012,342.631 salmon. In other applications, such as allocating 10 fishing boats, say, to perhaps three fishing grounds, the possibility of having non-integer answers may lead to erroneous conclusions and one of the integer programming techniques would then be most appropriate. Next, the deterministic nature of the LP approach is, of course, a deficiency in the probabilistic real world. The manager must realize that a full stochastic treatment of the salmon allocation as an optimization problem would most likely be a very difficult task. As alternates, an error structure could be applied to various elements in the problem, thus enabling one to explore a variety of probabilistic phenomena, or chance-constrained programming might be employed. Monte Carlo and simulation approaches might also be utilized but these are not per se optimization procedures. Finally,

the static nature of the LP approach provides a challenge to application in the sense that the unit values in the objective function, the constraints, and the right-hand sides of the constraint equations must not only be known in advance, but also must not change as a result of any of the allocations in the model.

The above assumptions can be handled in a variety of ways such as those indicated to handle problems of the deterministic nature. For example, we might, in some instances, use quadratic programming to handle the problem of non-linearity or dynamic programming or apply the outlined procedure in real time to handle the static nature of the programming problem, but unfortunately these approaches will present what can be quite complicated computational difficulties which may, in some instances, be insurmountable. It is thus clear that we have made certain approximations, trading off realism for an easily computable solution which certainly provides management guidance.

As we implied previously, we do not consider our departures from realism to seriously affect the utility of the model to provide guidance for decision making. Thus we believe that, for example, fixing the cannery capacity independent of the entities involved (or we could consider the cannery capacity to be fixed at a level which would accept a reasonable mixture of the entities) or using a simple average fecundity of the female entities to represent the average fecundity of the spawning females materially affects our conclusions. These, however, can be evaluated in direct applications by a sensitivity analysis.

Having outlined some cautions with respect to assumptions, we can now examine some of the indications provided by the various trials of the procedure. These involve the value of the fish on the dock, a reduction in processing-season length, changing value of entities during the run, and finally future data needs.

First with respect to the value of the total catch on the dock, we experimented with three value functions which set the daily value of each entity. Using the value functions to determine the value for each entity and day, and the actual distribution of the catch over the 1960 season, a total value of the catch was calculated which corresponds to the use of each of the three value functions. These values of the actual allocation of the catch were compared with the value of the optimal allocation as determined by the linear program as an indication of the value of optimally allocating the catch over the season. The increased value of the optimally allocated catch ranged from approximately $350,000 to $420,000 dependent on which value-function curve was considered. Table 3 shows these results. In the table, a fourth value function is indicated, which is simply a straight-line function such that the value of each entity remained constant through the season. Each of the other value functions was determined such that the average value of each curve was equal to the constant value for that entity for the season.

TABLE 3.—Comparison of the value of the optional allocation with the value of the actual allocation of the catch for the 1960 season.

	Value function 1[1]	Value function 2[2]	Value function 3[3]	Value function 4[4]
Optimal allocation	$13,787,050	$13,927,860	$13,792,555	$13,517,870
Actual allocation	13,378,650	13,506,250	13,439,825	13,517,890
Increased value	$ 408,400	$ 421,610	$ 352,730	$ [6] −20

[1] After day 6, the price dropped 3¢ per pound.
[2] The price was reduced by subtracting a logistic curve that reduced the price of each entity by 3¢ per pound over the season.
[3] The price was reduced by subtracting a quadratic curve that reduced the price of each entity by 3¢ per pound over the season.
[4] The price for each entity remained constant through the season (actual situation.)
[5] Difference due to rounding in the linear programming algorithm.

All three value functions had the effect of placing emphasis, in the optimal solution, on catching fish on the early days of the season. For two of the functions the value for any entity of fish on a given day is less than the value for that entity on the previous day. This is not true in the step function and thus we do not have a unique allocation, but rather a set of allocations under the high values and a set of allocations under the low values. But results are exactly the same; optimal allocations of fish are identical under the three value functions, although the total value of the catch changes somewhat, according to the exact shape of the value-function curve. Again, we emphasize that these gains from allocation can only be obtained by

knowing in advance of the run the information that we actually used in the allocation and having the ability to select the entities in the run as they are selected in the allocation.

Next, an examination of the 1960 optimal allocation reflects that this optimal allocation not only increases the value of the fish on the dock, it also shortens the length of time which a cannery needs to operate. Thus, the same amount of fish could be processed in a shorter period of time, by the same labor force, etc. In the optimal allocation for the 1960 run, all of the fish could have been processed in the first 13 days of the season, 5 days less than the actual operation. Naturally, we need to assume that a policy of catching salmon only from the early part of the run would not affect the genetic constituency of the stock. Furthermore, we must be careful here because, as we have emphasized in several places, by our LP assumptions, we cannot, a priori, let the cannery operations on day j-1, for example, affect the cannery operations on day j and we cannot at least in our formulation allow operating at peak capacity to affect quality of the fish or overtime payments since the variables are external to our model.

Another indication is that the values of fish change during the course of the season and that these values change in rather subtle ways depending upon the "rules" that we set forth (e.g., contrast Figures 8 and 9) and that in the fishery the marginal value of less valuable entities in Table 2 can be greater than the more valuable entities in Table 2. These changes in values need to be acknowledged in any management scheme.

Thus, it appears that we have the opportunity to increase the economic efficiency of some salmon runs. This is, of course, not a new concept, having been treated in some detail by, for example, Crutchfield and Pontecorvo (1969). Our approach is slightly different, however, in that we have concentrated on optimality only from the point of view of increasing the value, as we have defined it, of the fish on the dock. Any full treatment of the management problem must, of course, consider the distribution of fishing effort and its ancillary fishing and economic implications.

Now if we accept the premise that conservation is "optimum" allocation of resources in the times-space stream (*cf.* Crutchfield and Pontecorvo, 1969), and if we observe that mathematical programming provides guidance for optimal allocation, and note that LP is a special case of mathematical programming, and suggest that the kinds of information required to allocate salmon among the days of the run in an LP model are not going to be much different from the kinds of information required for more sophisticated programming procedure, then we are led to the conclusion that perhaps we have not addressed ourselves to asking, in our research, the "right questions" concerning salmon management. Following our argument, it would then be implicit that the right questions are contained in our formulation of the LP model. These answers must be feasible to obtain and they would contain either needed data or documented policies which would be reflected in the right-hand side of the constraint equations and, more importantly, provide an opportunity for enlightened dialogue. There is unfortunately a cost associated with asking right questions. This cost involves the cost of doing new work, or that which inevitably results when existing research activities are reallocated. Are these costs worth the expenditure? These, however, are the kind of questions, the answers to which can be guided by the LP problem. For the salmon management model, we impute values to units of cannery capacity, etc., but, and perhaps of equivalent importance, we impute a value, in meaningful terms, to information. Thus, for our salmon problem, we have cleverly avoided indicating how we could catch X_{ij} fish for some i,j. But it is well known that catching can be approximated because it is possible to catch salmon in traps (although this has never been done to any large extent in Bristol Bay) and, upon visual inspection, to distinguish between large and small, male and female fish, and doing this by virtue of *ceteris paribus*, the allocative process, we could add about 0.5 million dollars to the value of the salmon on the dock. This is, of course, not the full picture, because we would have to trade off the added value of salmon (it

is a common opinion that salmon caught in traps are of better condition and higher value than the salmon which are taken by gill netting, for example), the reduction in cannery days used to process the fish, the cost of building traps, and the political problems which are described in some detail in Crutchfield and Pontecorvo (1969). It would not, however, be difficult to determine the discounted present value of the various alternate procedures and thus evaluate the wisdom of engaging in any. In this evaluation, we need not be bound by what are perhaps extreme solutions such as traps, but we could examine the value of other selectivity procedures such as modifying gill net selectivity, etc. In general, then, we can evaluate the value of information by approximating that information, employing it in the model, and contrasting the change in the objective function with the objective function when the information is not in the model.

Additional information is needed on the pattern of the run. For the earlier years, this is available in Royce (1965), a publication which needs to be updated and implemented to obtain even rough estimates of the temporal movement of the fish of various entities through the fishery. This might be quite difficult to accomplish with present concepts, and the feasibility of a system which would acoustically monitor the passage of salmon through the entire river system and developing a central computer-oriented unit which would process the signals from all acoustic units and provide, in real time, through appropriate algorithms, rules for catching fish and making observations on escapement is presently being explored.

In our model, because of a lack of information, we used the total run and allocated this proportionately among the days of the fishery to determine the daily run. This emphasizes the need to have, for the purpose of management, a fairly accurate preseason guess of the total magnitude of the run and the X_{ij}'s. These guesses are already being made and the predictions need to be judged on the basis of whether the predictions do better than simply averaging the run for cycle years and simply averaging the run for noncycle years and applying these averages as predictions. The trick then may not be to estimate the average catch but rather to determine which years are cycle years.

We have included cannery capacity in a rather simple way in our model and this is a subject that also needs additional data since the cannery capacity constraint can be formulated in a variety of ways. It would be interesting to explore in a simulation setting the behavior of the slack variables in the cannery constraint. This is because it seems quite likely that there is a positive correlation between the cost of operating a cannery and the magnitude of the slack variable in the cannery constraint. If the run was constant from year to year, then it would be relatively easy to determine an optimal value for the magnitude of the slack variable in the cannery constraint. But the run varies considerably from year to year, and so in those years when the cannery constraint might be too low, we have an opportunity cost which appears as a slack variable in the dual formulation of the cannery constraint. It would seem then that the best value of the cannery constraint would be somewhere in between the capacity for a maximum run and a minimum run and that this might be investigated by employing the LP model in a simulation setting.

We have also employed egg and sex ratio constraints in our model. The egg constraints require information on fecundity and escapement. There is not much information on fecundity but this should be either easily obtainable or easily approximated. Again, the static nature of the LP problem makes it difficult to attribute a value to an egg for years in the future. This is, of course, important, emphasizing the need of thinking not, as is conventionally done, in terms of the forthcoming year, but rather in terms of, for example, a series of years maximizing (*cf.* Riffenburgh, 1969) economic benefits. In other words, the utilizers of resources may not be interested (even though they may think they are) in management on a year-to-year basis; rather, they are interested in some long-run satisfactory behavior of the time stream of economic benefits. Alternatively, though, we must be cautious of on-the-average management schemes which are typically presented in fishery appli-

cations. This is because a particular management scheme might be on-the-average quite profitable in the long run but might frequently completely bankrupt the system for the first 20 years of operation.

The problem of sex ratio is quite important because it appears that the objective function would be quite sensitive to selectively decreasing the number of males in the escapement and thus increasing the catch perhaps substantially. As indicated previously, Mathisen's study (1962) gives us some guidance on this subject and it would appear that, in some instances, the 3:1 ratio might be conservative. Furthermore, it should be mentioned that a year-to-year modification of sex ratio might be a useful cushion for approaching stability for some economic aspect of the fishery. Finally, the problem of escapement eludes us because in the wealth of literature on the subject there appears to be very little that is useful in setting the egg-minimum constraint. It is generally agreed that the stock-recruitment relation for salmon is the familiar Ricker-type curve. It is well known that the variability in these relations is quite large (in the case of the Naknek-Kvichak run, attempting to draw similarities between stock and recruitment places tremendous stresses on the imagination anyhow) and as a consequence, if the dome-shaped model holds, a minimum escapement set sufficiently, but not unreasonably high, could, on the average be reducing the return rather than increasing the return.

It might be difficult even after several years of setting the minimum escapement value at too high a level, to detect, owing to the variability in the system, the effect of this policy. If this is true, then again we are asking the wrong questions by studying the stock and recruitment model per se. We are faced with a system that is so variable, either intrinsically or in terms of measurement techniques, or both, that a large number of data points is required before we can evaluate the relation between the empirical data and the theory and then use the theory to predict. There is but one point a year and so we are asking nature to "stand still" for a large number of years. Given these observations and our past experience, we wonder whether it might not be more appropriate for management purposes to avoid looking at stock and recruitment per se, to intensify study of the physiology and behavior of very young stages of fish, and thus examine fundamental problems of cause and effect, vis-á-vis the variables that influence the magnitude of egg production and survival of these eggs and larvae or other young stages through the first several months of their life. And finally, in the meantime, would it be more appropriate to consider measuring stock and recruitment in terms of transition probabilities which might be estimated by computing the median stock and the median recruitment? Stock sizes which are below the median would be poor, those which are above, good, and similarly with recruitment. The empirical data could then be used to estimate probabilities of good-good, good-poor, poor-good, and poor-poor transitions. We need not in this procedure be restricted to medians, but could in fact use any fractile, and in fact we need not be restrained by fractiles because we might want to place the dividing line at some "optimal value" and explore the consequences.

In conclusion, then, we have formulated a LP model for salmon runs and have shown how it might be related to the Naknek-Kvichak run. We see in this relationship that, given information on the structure of the run, we can both increase the value of the fish on the dock and at the same time reduce processing time. Whether it is worth obtaining the information in terms of the indicated data and the ability to select fish from the run to approach this allocation and whether decreased processing time is, in fact, a saving, are questions that must be answered by the processing industry in light of the increased value of salmon on the dock. If our estimate of increased value is approximately correct, we can see that allocation can add an interesting value to the catch, but far greater additions could come from reducing the escapement, if this is possible, and alleviating the open-access related problems. Perhaps the most interesting feature of the model is the richness of interpretations that LP affords in the salmon situation and the nature of questions and data needs raised by the model. Finally, we emphasize that, as Hillier and Lieberman (1967) note,

"A practical problem which completely satisfies all of the assumptions of LP is very rare indeed. However, the LP model is often the most accurate representation of the problem, which will yield a reasonable recommendation for action before implementation is required."

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APPENDIX TABLE 1.—Constants used in value-function equations for each entity and day of the 1960 run.

		Function 1[1]	Function 2[2]	Function 3[3]
Entity 1	IP_1	1.382	1.365	1.331
	b_1	.153	.153	.000417
Entity 2	IP_2	1.998	1.974	1.925
	b_2	.222	.222	.000583
Entity 3	IP_3	1.470	1.455	1.425
	b_3	.135	.135	.000472
Entity 4	IP_4	1.964	1.944	1.903
	b_4	.186	.186	.000694

[1] For entity i on day j,
Value $_{i,j} = IP_i$, for $j < 6$. Value $_{i,j} = IP_i - .03X$ (weight of entity i in pounds), for $j > 6$

[2] For entity i on day j,
$$\text{Value}_{i,j} = IP_i - \frac{j_i}{1+e^{-(4.5\ +\ 5j)}}$$

[3] For entity i on day j,
Value $_{i,j} = IP_i - b_i X^2$

APPENDIX TABLE 2.—Total run, total catch, and value functions for each entity and day of the 1960 season.

Entity	Day	Total run	Total catch	Value function 1[1]	Value function 2[2]	Value function 3[3]	Entity	Day	Total run	Total catch	Value function 1[1]	Value function 2[2]	Value function 3[3]
1	1	280,351	119,149	1.382	1.362	1.331	3	1	211,080	53,192	1.470	1.453	1.425
	2	368,062	156,426	1.382	1.361	1.329		2	277,120	69,834	1.470	1.451	1.423
	3	475,118	201,925	1.382	1.358	1.327		3	357,724	90,146	1.470	1.449	1.421
	4	600,110	255,046	1.382	1.353	1.323		4	451,832	113,861	1.470	1.445	1.418
	5	737,489	313,432	1.382	1.347	1.319		5	555,267	139,927	1.470	1.439	1.415
	6	876,389	372,465	1.382	1.337	1.314		6	659,846	166,281	1.470	1.430	1.410
	7	1,000,833	425,354	1.229	1.324	1.308		7	753,542	189,892	1.335	1.419	1.405
	8	1,092,325	464,238	1.229	1.307	1.301		8	822,427	207,252	1.335	1.404	1.398
	9	1,134,780	482,281	1.229	1.288	1.293		9	854,393	215,307	1.335	1.387	1.391
	10	1,120,032	476,014	1.229	1.270	1.284		10	843,289	212,488	1.335	1.371	1.383
	11	1,050,967	446,661	1.229	1.253	1.274		11	791,289	199,404	1.335	1.356	1.375
	12	940,395	399,668	1.229	1.240	1.264		12	708,038	178,426	1.335	1.345	1.365
	13	806,344	342,696	1.229	1.230	1.251		13	607,110	152,992	1.335	1.336	1.355
	14	666,514	283,268	1.229	1.224	1.238		14	501,829	126,460	1.335	1.330	1.343
	15	534,438	227,136	1.229	1.219	1.225		15	402,387	101,401	1.335	1.326	1.331
	16	418,185	177,729	1.229	1.216	1.210		16	314,858	79,344	1.335	1.324	1.318
	17	321,003	136,426	1.229	1.215	1.195		17	241,688	60,905	1.335	1.322	1.304
	18	242,797	103,188	1.229	1.214	1.178		18	182,805	46,066	1.335	1.321	1.290
2	1	9,590	5,427	1.998	1.970	1.924	4	1	24,985	17,289	1.964	1.941	1.902
	2	12,590	7,126	1.998	1.967	1.922		2	32,803	22,700	1.964	1.939	1.901
	3	16,252	9,199	1.998	1.963	1.919		3	42,344	29,302	1.964	1.935	1.898
	4	20,528	11,618	1.998	1.957	1.914		4	53,484	37,010	1.964	1.930	1.894
	5	25,227	14,278	1.998	1.948	1.908		5	65,727	45,483	1.964	1.922	1.888
	6	29,978	16,967	1.998	1.934	1.900		6	78,106	54,050	1.964	1.910	1.882
	7	34,235	19,377	1.776	1.914	1.891		7	89,197	61,724	1.778	1.894	1.874
	8	37,365	21,149	1.776	1.890	1.881		8	97,351	67,367	1.778	1.874	1.866
	9	38,817	21,970	1.776	1.863	1.869		9	101,135	69,985	1.778	1.851	1.856
	10	38,313	21,968	1.776	1.836	1.856		10	99,820	69,075	1.778	1.828	1.845
	11	35,950	20,347	1.776	1.812	1.841		11	93,665	64,816	1.778	1.808	1.832
	12	32,168	18,207	1.776	1.792	1.825		12	83,811	57,997	1.778	1.792	1.819
	13	27,582	14,479	1.776	1.778	1.808		13	71,864	49,730	1.778	1.780	1.804
	14	22,800	12,905	1.776	1.769	1.789		14	59,402	41,106	1.778	1.772	1.789
	15	18,281	10,347	1.776	1.763	1.769		15	47,631	32,960	1.778	1.767	1.772
	16	14,305	8,096	1.776	1.759	1.747		16	37,270	25,790	1.778	1.763	1.754
	17	10,980	6,215	1.776	1.756	1.724		17	28,609	19,797	1.778	1.761	1.735
	18	8,305	4,700	1.776	1.754	1.700		18	21,639	14,974	1.778	1.760	1.714

[1] After day 6, the price dropped 3¢ per pound.
[2] The price was reduced by subtracting a logistic curve that reduced the price of each entity by 3¢ per pound over the season.
[3] The price was reduced by subtracting a quadratic curve that reduced the price of each entity by 3¢ per pound over the season.

CHEMICAL AND NUTRITIONAL CHARACTERISTICS OF FISH PROTEIN CONCENTRATE PROCESSED FROM HEATED WHOLE RED HAKE, *Urophycis chuss*

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ABSTRACT

This study was to determine whether cooking lean, whole fish before they are extracted by solvent affects the chemical and nutritional characteristics of the resulting fish protein concentrate. When red hake were heated at 100° and 109° C for as long as 80 min, the chemical and nutritional properties of the fish protein concentrate were not adversely affected significantly. The nutritional quality was slightly lower, however, in fish protein concentrate produced from red hake that were heated at 121° C for 10 to 80 min.

Fish protein concentrate (FPC) contains protein that is high in quality. It therefore can be used to supplement diets that contain inadequate amounts of high-quality protein.

Fish protein concentrate is prepared by removing most of the lipids and water from whole fish. Several methods for preparing FPC have been investigated. They can be classified as chemical, biological, and physical. Most investigators have used chemical methods in which solvents extract the lipids and water from whole fish.

In the United States, two processes for making FPC have been approved by the Food and Drug Administration. Both of these are chemical processes in which solvents are used. In the overall program of the National Marine Fisheries Service National Center for Fish Protein Concentrate, various approaches to processing are being investigated. One such approach is cooking and pressing fish prior to solvent extraction. This procedure would tend to reduce the volume of solvent required for extraction, inasmuch as water and lipids would be expressed during the pressing stage.

Raw fish are difficult to press because of their physical consistency. The processor can overcome this problem by cooking the fish before pressing them. If he subjects the fish to a high temperature for a long time, however, undesirable chemical reactions may occur that decrease the quality of the protein.

The purpose of this study therefore was to find whether or not the chemical composition and nutritional quality of fish protein concentrate are altered when the FPC is produced by solvent extraction of fish that have been cooked at different temperatures for varying periods of time.

CHEMICAL COMPOSITION

Reported here are both the proximate composition and amino acid composition of the FPC produced from cooked fish.

PROXIMATE COMPOSITION

We used red hake, *Urophycis chuss*, which are lean fish. They were caught off the coast of New England in the area of Block Island, situated south southwest of Point Judith, R.I. The hake were iced on board the vessel and were then frozen in 25-lb. wax laminated cartons at the dock. The hake were kept frozen while being shipped to the National Marine Fisheries Service National Center for Fish Protein Concentrate in College Park, Md. The shipment contained about 96 cartons. From these 96 boxes, 15 cartons (375 lb.) were picked at random for the investigation and were stored

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at —20° C (The other cartons were used in another experiment.) The hake were used within 1 month after storage.

About 17 to 18 hr before studying each processing variable, we placed one carton of fish in a refrigerated room at a temperature of 5° to 6° C. This treatment allowed the fish to thaw sufficiently so that they could be handled individually. The fish were ground through a Hobart meat grinder,[2] which was equipped with an end plate containing holes that were one-quarter inch in diameter. After the hake were ground, they were thoroughly mixed, and a sample that weighed 20 lb was removed. The sample was divided into three equal portions, and each portion was placed in a 2-inch-deep tray lined with aluminum foil. (This procedure was used in order to permit existing equipment to be used.)

The trays were placed in an autoclave and were heated at 100°, 109°, or 121° C for 10, 20, 40, or 80 min. Thermocouples were used to measure the temperature of the samples. After being heated, the trays were removed from the autoclave, were covered with aluminum foil, and were cooled in a refrigerated room at 5° to 6° C. A control sample was also prepared, which consisted of raw, unheated ground hake.

The entire contents of the trays were mixed with solvent at a 2:1 (w/w) ratio of solvent to solid. The samples were extracted by the "cross-current" batch-extraction procedure described by Brown and Miller (1969). The solvent used for extraction was 91%, by volume, isopropyl alcohol.

The extracted and dried samples of FPC were ground in a Rietz Disintegrator.

The samples were analyzed for crude protein, volatiles, and ash by the methods described by Horwitz (1965). Lipids were determined by the method of Smith, Ambrose, and Knobl (1964).

Table 1 shows the concentrations of crude protein, ash, and lipids found.

The concentration of crude protein in the samples that were heated was slightly lower than in the sample that was not heated. The concentration of crude protein, however, was not significantly affected by the temperature at which the samples were heated. Also, the time of heating did not significantly affect the concentration of crude protein in the samples, except for the 20-min treatment. The samples that were heated for 20 min had a slightly lower concentration of crude protein than did those that were heated for the other intervals of time.

TABLE 1.—Proximate composition, expressed on a moisture-free basis, of samples of FPC prepared from hake that were heated for varying times and temperatures before being extracted with solvent.

Sample	Crude protein	Ash	Lipid
	%	%	%
Nonheated control	88.7	13.8	0.16
Heated samples:			
100° C for:			
10 min	86.0	15.3	0.16
20 min	85.3	15.4	0.16
40 min	86.3	15.0	0.20
80 min	85.9	15.5	0.15
Mean	85.9	15.3	0.17
109° C for:			
10 min	87.5	14.4	0.12
20 min	85.6	15.8	0.12
40 min	86.8	15.7	0.21
80 min	87.4	13.8	0.15
Mean	86.8	14.9	0.15
121° C for:			
10 min	87.3	13.6	0.12
20 min	85.9	15.2	0.14
40 min	86.5	13.7	0.16
80 min	86.8	14.3	0.16
Mean	86.6	14.2	0.14

The concentration of ash was slightly higher in the samples that were heated than in the sample that was not heated. The concentration of ash in the heated samples was not affected by either the temperature of heating or the length of time of heating.

The concentration of lipid in the samples was somewhat variable, but it was not significantly affected by the treatments.

AMINO ACID COMPOSITION

Essential amino acids, except for tryptophan, were determined with an automatic amino acid analyzer by the method described by Moore, Spackman, and Stein (1958). Tryptophan was determined chemically by the method of Spies and Chambers (1949). Cystine was determined

[2] The use of trade names is merely to facilitate descriptions; no endorsement is implied.

microbiologically by the method of Henderson and Snell (1948). Available lysine was determined by the method described by Carpenter (1960).

We found only slight changes in the concentrations of amino acids in the samples (table 2). The treatments that we used did not consistently affect the concentrations of amino acids, except for cystine and available lysine. The concentration of cystine was reduced in the sample heated at 121° C for 80 min. The concentrations of available lysine were slightly lower in the samples heated at 100° and 109° C for 20 min. The reason for these decreases is not apparent to us.

Evans and McGinnis (1948) previously reported that cystine was reduced when soybeans were autoclaved at 130° C for 60 min.

NUTRITIONAL QUALITY

The nutritional quality of the samples was determined in a feeding study using rats. Diets were prepared that contained 10% protein from the heated samples, the nonheated sample, or casein. The diet that contained the nonheated sample served as a control, and the one that contained casein served as a reference standard. The composition of the basal diet was described earlier by Stillings, Hammerle, and Snyder (1969).

Male weanling rats of the Carworth Farms CFE strain were received when they were 22 days old. The rats were housed individually in cages with screen bottoms and were kept in an air-conditioned room maintained at about 23° C. During the first 2 days, the rats were fed a basal diet containing 15% casein. They were then allotted to groups on the basis of weight, and the groups were randomly assigned to different diets. Each group contained 10 rats, and the rats were offered feed and water *ad libitum* for 4 weeks.

The amount of feed consumed was recorded three times each week, and the gains in weight were determined once each week. At the end of the experiment, the protein efficiency ratio was determined by dividing the gain in weight by the weight of protein consumed.

The data were analyzed statistically. Differences between means were determined by Tukey's procedure as described by Steel and Torrie (1960: 109).

Table 3 shows the data on the nutritive quality of the FPC samples. Based on the gain in weight, intakes of feed, and protein efficiency ratios, the quality of the samples that were heated at 100° and 109° C was not significantly different from the quality of the control, which was not heated. Samples that were heated at 121° C, however, had a lower quality than the control sample. In general, the quality of the samples heated at 100° and 109° C was equal to that of casein or was slightly higher than

TABLE 2.—Amino acid composition of FPC samples prepared from hake that were heated for varying times and temperatures before being extracted with solvent.

Amino acid	Concentration of amino acid in:											
	Unheated control sample	Samples heated at:										
		100° C for:				109° C for:				121° C for:			
		10 min.	20 min.	40 min.	80 min.	10 min.	20 min.	40 min.	80 min.	10 min.	20 min.	40 min.	80 min.
							Grams per 16 grams of nitrogen						
Arginine	6.2	6.7	6.4	6.8	6.4	5.7	7.0	6.1	6.4	6.9	6.1	6.6	6.3
Histidine	1.8	2.0	1.9	1.9	1.8	1.7	1.9	1.7	1.8	2.1	1.9	1.8	2.0
Isoleucine	4.5	4.3	4.4	4.5	4.3	4.3	4.6	4.5	4.5	4.9	4.6	4.4	4.8
Leucine	7.4	7.2	6.9	7.1	6.9	7.0	7.5	7.0	7.2	8.0	7.2	7.3	7.6
Lysine	7.7	8.3	7.8	8.0	7.4	7.1	7.7	7.1	7.5	8.4	7.5	7.9	7.6
Methionine	3.2	3.1	3.1	3.1	3.0	3.0	3.0	3.1	3.0	3.6	3.1	3.1	3.4
Phenylalanine	4.1	4.1	4.0	4.1	4.0	4.0	4.2	4.1	4.1	4.5	4.1	4.2	4.3
Threonine	4.3	4.2	4.1	4.2	4.0	4.0	4.4	4.2	4.0	4.6	4.1	4.2	4.4
Tryptophan	1.0	1.2	1.2	1.0	1.3	1.2	1.1	1.1	1.2	1.1	1.2	1.3	1.2
Valine	5.1	4.9	5.0	5.0	5.1	4.8	5.2	4.6	5.0	5.5	5.0	5.0	5.3
Cystine	0.9	1.2	1.2	1.2	0.9	1.1	1.1	1.1	1.1	1.1	1.1	--	0.7
Available lysine	7.9	7.8	6.9	7.2	7.9	7.7	6.9	7.2	7.6	7.8	7.1	7.2	7.6

TABLE 3.—Weight gain, feed intake, and protein efficiency ratio of groups of 10 rats fed diets of FPC samples prepared from red hake that were heated for varying times and temperatures before being extracted with solvent.

Sample	Average daily weight gain	Average daily feed intake	Protein efficiency ratio
	Grams	*Grams*	
Nonheated control	4.85	13.8	3.37
Heated samples:			
100° C for:			
10 min	4.96	14.0	3.41
20 min	4.37	13.0	3.25
40 min	4.50	13.1	3.37
80 min	4.29	13.3	3.16
Mean	4.53	13.4	3.30
109° C for:			
10 min	4.37	12.7	3.35
20 min	4.31	13.1	3.11
40 min	4.45	13.4	3.20
80 min	4.20	12.6	3.28
Mean	4.34	13.0	3.24
121° C for:			
10 min	3.21	10.6	3.01
20 min	4.01	12.2	3.13
40 min	3.52	11.1	3.10
80 min	3.20	10.3	3.02
Mean	3.49	11.0	3.07
Casein	3.85	12.0	3.18
Tukey's W ($P<0.05$)	0.81	2.0	0.26

that of casein. When the temperature was increased to 121° C, the quality of the samples was slightly lower than that of casein but not significantly so. At each temperature, the temperature at which the samples were heated had a more significant effect on the quality of the samples than did the length of time of heating.

SUMMARY AND CONCLUSIONS

We conducted a study to determine the chemical composition and nutritional quality of FPC produced from fish that are heated before they are extracted with solvent. Red hake, which are lean fish, were heated at 100° C for 10, 20, 40, or 80 min. Other samples were heated for these same lengths of time at 109° or 121° C. The samples were then extracted with isopropyl alcohol. The FPC produced from the samples of hake that were heated contained slightly less crude protein and more ash than did the FPC produced from the samples that were not heated. The amino acid composition of samples that had been heated did not differ markedly from the composition of those that were not heated. The nutritive quality of the samples that were heated at 100° and 109° C was not significantly affected. Samples heated at 121° C, however, were lower in quality than was the control sample.

We conclude that red hake can be heated at temperatures of 100° and 109° C for as long as 80 min before being extracted by solvent without the quality of the protein being affected significantly.

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EFFECT OF ICE STORAGE ON THE CHEMICAL AND NUTRITIVE PROPERTIES OF SOLVENT-EXTRACTED WHOLE FISH—RED HAKE, *Urophycis chuss*

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ABSTRACT

Because red hake that are to be used in the future production of fish protein concentrate will be caught in quantity, the preservation of the hake during periods of glut will present a problem that possibly can be solved by storage of the hake in ice.

In our study of this problem, whole red hake were held in ice for 2, 6, 8, and 11 days. Organoleptic tests on the fresh fish showed that they were edible on the 8th day but were not edible on the 11th day.

Samples of fish were removed during each period of storage and were processed (1) by freeze-drying to produce a reference sample (2) by solvent extraction with isopropyl alcohol to produce a fish protein concentrate. Proximate composition, amino acid composition, and nutritive quality were determined comparatively on both of these two kinds of processed samples.

From the data obtained, we concluded that red hake stored in ice for 8 days are suitable for use in the production of fish protein concentrate and that they would be suitable for this use up to the point of spoilage of the fish, which occurs sometime between 8 and 11 days.

In the period between the capture and processing of fish that are to be used in products for human consumption, they must be preserved in a manner that maintains their food-grade quality. This requirement applies to the production of fish protein concentrate (FPC) as well as to that of more common fish products.

The preservation of fish is a problem not only aboard the harvesting vessel but at the shore processing plant as well. The problem ashore becomes especially important during periods of glut when the fresh fish must be held several days before being processed.

In the manufacture of FPC by the method we use, oil and moisture are removed from the fish with isopropyl alcohol. We therefore investigated the possibility of holding fish in this solvent (Dubrow and Hammerle, 1969). We found the method to be entirely suitable for periods of holding up to 11 days.

Although storage in isopropyl alcohol was satisfactory, more conventional means of holding the fish, such as storing them in ice, are likely to be used in commercial operations. During the time fish are held in ice, however, considerable change may occur in the components of the fish tissue. Endogenous and bacterial enzymes may break down protein into water-soluble and volatile components, causing off-flavors and odors in the fish. In addition, the highly unsaturated lipids of the fish may oxidize rapidly, causing the fish to become rancid.

While these changes are taking place in iced fish, the water from the melting ice is leaching out some of the compounds that are forming. Furthermore, the subsequent extraction with alcohol during the production of FPC, if adequate, removes most of the undesirable compounds that were not leached out by the melt water.

Just what effect the enzymatic and oxidative changes have on the various components of the tissues as well as on the nutritive quality of the protein in the finally processed FPC is not known. Accordingly, solubilization of the components of the fish tissues could alter the composition of the finally processed FPC. We should

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know, of course, what occurs, because FPC is of value solely as a protein supplement of high quality.

The aim of this study therefore was to determine the effect that storage of food-grade fish in ice has on the chemical composition of the components of the tissue and on the nutritive quality of the protein. We accomplished this aim by comparing FPC made from samples of the ice-stored fish with reference samples made by freeze-drying samples of the fish. We used freeze-drying because we believe that this method of production results in minimum alteration in the samples during drying.

CHEMICAL COMPOSITION

Both the proximate composition and the amino acid composition of the samples were determined.

PROXIMATE COMPOSITION

As indicated earlier, we used standard reference samples produced under ideal conditions, as a basis on which to evaluate our samples of FPC.

Standard Reference Sample

About 600 lb of red hake were caught on January 6, 1965, in 25 to 26 fathoms of water off the coast of Rhode Island. The fish were divided randomly into lots of 100 lb each, were iced immediately, and then were taken to the Bureau of Commercial Fisheries (BCF) (now National Marine Fisheries Service) Technological Laboratory at Gloucester, Mass., where they were held in ice.

During the next 11 days, each lot of fish was inspected periodically for freshness by experienced BCF fish inspectors at Gloucester. The factors they considered were (1) damage to the fish, (2) conditions of the skin, eyes, and gills, and (3) texture, odor, and flavor of cooked samples. A numerical score ranging from one to four was used to rate fish of varying quality for each of the factors. Fish of perfect or nearly perfect quality were assigned a value of 1, whereas those at the limit of acceptability or beyond the limit were assigned a value of 4.

Table 1 shows the data on the subjective evaluation of the raw fish. The samples of fish tested after storage for 11 days in ice were judged to be at the limit of acceptability. The fish that had been stored in ice for 8 days were of acceptable quality and were considered to be of food grade.

TABLE 1.—Freshness evaluations of raw red hake stored in ice for periods up to 11 days.
[Each sample had 50 fish.]

Storage time	Average subjective evaluations of:						
	Damage	Skin	Eyes	Gills	Texture	Odor	Flavor
Days							
2	1.00	1.00	1.02	1.40	1.50	1.08	1.0
6	2.26	2.22	2.04	2.32	2.60	2.38	2.0
8	2.44	2.50	3.00	2.92	3.18	3.10	2.5
11	3.10	4.00	3.86	3.92	3.98	4.00	--

Fish of perfect or nearly perfect quality were assigned a value of 1; those of unacceptable quality were assigned a value of 4.

After the iced fish had been inspected for quality, they were shipped in ice to College Park, Md. Each box of fish, upon receipt at College Park, was divided into two groups and were processed immediately—one into a standard reference sample and the other into FPC.

One portion of 20 lb was selected at random from the group of fish to be used as a standard reference sample. The standard reference sample was prepared by freezing the fish in liquid nitrogen and grinding the whole fish through a Rietz Disintegrator[2] under a stream of liquid nitrogen, and then freeze-drying the liquid-nitrogen slurry of ground fish. The freeze-drying step was carried out under a pressure of 500μ of mercury and at a platen temperature of 40° C. The dried samples were then removed from the freeze dryer in an atmosphere of nitrogen and were sealed in containers. The containers were maintained at —40° C until the samples were needed.

The freeze-dried samples were analyzed for crude protein, ash, and volatiles in accordance with standard procedures (Horwitz, 1965). Total lipids were determined by the method of Smith, Ambrose, and Knobl (1964).

Table 2 shows the proximate composition of

[2] The use of trade names is merely to facilitate description; no endorsement of products is implied.

TABLE 2.—Proximate composition of freeze-dried, ground whole hake (standard reference samples) stored in ice for periods up to 11 days.

Storage time	Volatiles	Lipids[1]	Ash[1]	Crude protein[2]
Days	*Wt. %*	*Wt. %*	*Wt. %*	*Wt. %*
2	3.80	15.30	13.44	74.47
6	2.49	14.06	12.84	77.34
8	2.46	14.36	12.43	77.38
11	4.70	15.07	12.49	77.01

[1] The data on lipids, ash, and protein were based on the dry weight of sample.
[2] Crude protein was calculated as N X 6.25.

the various samples of freeze-dried whole fish. Data are presented on a dry-weight basis to reveal possible losses during storage.

The concentration of lipid varied between 14 and 15%; that of ash, between 12 and 13%. The data indicate that the nitrogen fraction did not change greatly. The crude protein remained relatively constant at about 77% (on a dry-weight basis) except on the second day of sampling. This deviation on the second day was probably the result of a sampling error. Analyses for nonprotein nitrogen would have been helpful for interpretive purposes. Unfortunately, they were not made. Dassow[3] has reported that the nonprotein nitrogen fraction of whole Pacific hake stored in ice did not change significantly over a period of 11 days.

Fish Protein Concentrate

From the remaining portion of each lot of fish, 20 lb were selected at random and were extracted with isopropyl alcohol according to established procedures (Brown and Miller, 1969). In brief, the fish were ground through a Hobart meat grinder, were slurried with 15 liters of 91% (v/v) isopropyl alcohol for 30 min, and were centrifuged. The centrifuged solids were then extracted continuously with hot isopropyl alcohol at 60° to 70° C and at a rate of flow of 0.2 gal per minute. After 2 hr the solids were removed by centrifugation and were desolventized under vacuum at 60° C.

[3] Dassow, John A. 1966. Statement of project accomplishment, Utilization of fishery resources program. *In* Quarterly progress report of the BCF Technological Laboratory, Seattle, Wash., July 1 - September 30, 1966. Unpublished report, 6 p.

This method of processing was not intended to be representative of commercial methods. It was used in our laboratory at that time solely as an experimental technique to evaluate selected variables in the preparation of FPC by solvent extraction. It has since been replaced by a several-stage countercurrent extraction system, which is both much more economical in the volume of solvent needed and is more representative of commercial processing methods. A comparison of FPC made by each system has shown no significant differences, however, either in chemical composition or in nutritive value.

The proximate composition was determined by the same method used with the freeze-dried fish.

Table 3 lists the proximate compositions of the FPC's prepared from the fish stored in ice for various periods. The concentrations of lipids and volatiles remained essentially unaffected by storage. The concentration of ash increased, however, and that of protein (that is, of nitrogen) decreased. The major change occurred after the 8th day of storage. Because the concentration of protein in the standard reference samples did not drop in the same manner as the concentration of protein did in the FPC's, the loss of protein could not have occurred during storage but must have occurred during processing. This conclusion could be accounted for by the formation, during storage, of soluble nitrogenous products resulting from enzymatic breakdown or bacterial breakdown, or from both, that were not leached out of the fish during storage but that were subsequently leached out during the extraction process used in making the FPC. This conclusion was further support-

TABLE 3.—Proximate composition of FPC prepared from raw fish stored in ice for periods up to 11 days.

Storage time	Volatiles	Lipids[1]	Ash[1]	Crude protein[2]
Days	*Wt. %*	*Wt. %*	*Wt. %*	*Wt. %*
2	4.25	0.18	12.30	89.70
6	5.10	0.13	12.44	89.65
8	5.12	0.10	13.19	89.30
11	4.10	0.21	16.04	86.94

[1] The data on lipids, ash, and protein were based on the dry weight of sample.
[2] Crude protein was calculated as N X 6.25.

ed by the observed decrease in yield after processing—namely, 12.0 percent of 2-day-old fish to 10.0 percent of 8-day-old fish.

Storage of whole red hake in ice up to 11 days did not influence the extractability of the lipids. A slight loss of nitrogen occurred, however, during the processing of whole fish stored for 11 days as compared with fish stored for shorter periods.

AMINO ACID COMPOSITION

Standard Reference Sample

Amino acids were determined by the method of Spackman, Stein, and Moore (1958).

Table 4 shows that the recovery of amino acids was relatively constant at about 92% of the protein. The essential amino acids for which analyses were made ranged between 45.5 and 46.3% of the total. No major change in the pattern of any one particular amino acid resulted from storage.

In general, this finding agrees with those by Cohen and Peters (1963) on whiting, *Merluccius bilinearis*, that were stored in ice. These authors reported, however, that methionine decreased after the 13th day with a subsequent increase in methionine sulfoxide. We do not know whether this compound was present in the hake that we studied.

Fish Protein Concentrate

The same methods were used as with the standard reference sample. That is, the amino acids were determined by the method of Spackman, Stein, and Moore (1958).

Table 5 shows the concentration of amino acids in the FPC's processed from the fish held in ice. The data indicate that about 100% of the amino acids were recovered.

The essential amino acids constituted 47% of the total amino acids in the FPC made from fish stored 2 days, but the concentration of these amino acids dropped to 43% after the fish had been stored 11 days. Individual amino acids decreased in concentration. Of these amino acids, leucine and isoleucine decreased slightly, whereas lysine and histidine decreased markedly after the 8th day of storage. The total concentration of lysine was about 11% less in the FPC made after the fish had been stored for 11 days than in the FPC produced after they had been stored for 2 days. The concentration of histidine

TABLE 4.—Amino acid composition of raw freeze-dried whole ground fish. The samples were prepared after fish were held in ice for periods up to 11 days.

Amino acid	Concentration of the given amino acid in the Standard Reference Samples after they were held:			
	2 days	6 days	8 days	11 days
	— — — *Percent of the protein (N X 6.25)* — — —			
Lysine	7.63	7.44	7.72	7.56
Histidine	1.92	1.77	1.92	1.76
Ammonia	1.68	1.57	1.58	1.59
Arginine	5.96	5.82	6.05	5.76
Aspartic acid	9.50	9.41	9.45	9.53
Threonine	4.07	4.16	4.08	4.14
Serine	4.08	4.22	4.11	4.11
Glutamic acid	14.05	14.32	14.27	14.23
Proline	4.57	4.79	4.69	4.76
Glycine	7.70	8.23	7.68	7.52
Alanine	6.50	6.56	6.36	6.41
Valine	4.88	4.78	4.63	4.98
Methionine	3.02	3.00	2.91	3.05
Isoleucine	4.21	4.17	4.13	4.32
Leucine	7.07	7.03	7.06	7.21
Tyrosine	3.01	2.90	2.88	2.99
Phenylalanine	3.82	3.98	3.98	3.94
Total amino acid recovery	91.99	92.58	91.92	92.27
Percent essential amino acids	46.29	45.51	46.21	46.30

TABLE 5.—Amino acid composition of FPC prepared from raw fish held in ice for periods up to 11 days.

Amino acid	Concentration of the given amino acid in the samples extracted after they were held for:			
	2 days	6 days	8 days	11 days
	— — — *Percent of the protein (N X 6.25)* — — —			
Lysine	8.67	8.14	8.38	7.72
Histidine	2.06	1.88	2.00	1.74
Ammonia	1.47	1.57	1.39	1.44
Arginine	7.12	6.98	7.24	6.92
Aspartic acid	10.36	10.36	10.17	10.00
Threonine	4.45	4.48	4.51	4.44
Serine	4.60	4.59	4.70	4.75
Glutamic acid	15.47	15.57	15.34	15.27
Proline	5.01	5.64	5.59	6.47
Glycine	8.04	9.20	9.12	10.23
Alanine	6.78	7.10	6.96	7.23
Valine	5.14	5.24	4.95	4.90
Methionine	3.32	3.32	3.46	3.29
Isoleucine	4.52	4.46	4.37	4.26
Leucine	7.70	7.54	7.44	7.17
Tyrosine	3.39	3.31	3.28	3.16
Phenylalanine	4.12	4.05	4.07	3.91
Total amino acid recovery	100.75	101.86	101.58	101.46
Percent essential amino acids	47.00	45.25	45.70	43.71

decreased about 15.5% within the same period of time. Both glycine and proline increased in percentage of the total amino acid concentration. This increase could possibly be due to the lack of enzymatic breakdown of the fish collagens, thereby increasing the percentage of these amino acids as compared with that of the amino acids of the myofibrillar proteins.

In retrospect, an analysis of the raw, unprocessed fish for free amino acids or total nonprotein nitrogen would have made the interpretation of these results more certain.

No marked differences in the amino acid pattern of the standard reference sample could be detected after storing the whole fish in ice for periods up to 11 days. The amino acid pattern of the FPC's produced from the same batch of fish as was the standard reference sample, did, however, show changes, which were more pronounced in the FPC processed from 11-day-old fish. These changes appeared to be the result of alcohol extraction of solubles that were apparently formed during ice storage and not leached out by the melt water from the ice.

PROTEIN QUALITY

STANDARD REFERENCE SAMPLE

Protein efficiency ratios were determined by the method of Campbell (1960). Diets of the standard reference samples and of FPC prepared from raw fish stored in ice were fed *ad libitum* to male albino rats (Charles River strain), which were randomly allotted to groups of 10 animals. The samples were added to a basal diet at a 10% level of crude protein. Gain in weight and consumption of food were recorded each week for 4 weeks, and the protein efficiency ratio was calculated as (weight gain)/(weight of protein consumed). A diet in which casein was the source of protein was used as a reference.

Table 6 shows the data obtained from the animal-feeding studies comparing the quality of the protein of the various samples. Except for the sample prepared from fish held 11 days, the protein quality of the standard reference samples was better than that of casein. The protein quality of the standard reference sample taken on the 11th day was similar to that of casein and therefore was lower than that of the three samples taken earlier.

TABLE 6.—Mean weight gained, food consumed, and adjusted protein efficiency ratio of groups of eight rats fed freeze-dried whole hake prepared from fish stored in ice, compared with casein.

Storage time	Mean weight gained	Mean weight of food consumed	Adjusted protein efficiency ratio[1]
Days	*Grams*	*Grams*	
2	158.6 ± 3.16	390 ± 5.7	3.46 ± .05
6	150.8 ± 3.08	385 ± 5.9	3.35 ± .08
8	155.6 ± 5.28	381 ± 7.7	3.49 ± .07
11	148.6 ± 3.35	400 ± 3.9	3.18 ± .07
Casein	113.5 ± 5.65	323 ± 3.9	3.00 ± .00

[1] The protein efficiency ratios were adjusted to a protein efficiency ratio of 3.00 for casein.

Proximate composition and concentrations of amino acid do not account for the difference obtained in the quality of the protein in the sample of fish held in ice for 11 days. Because the fish were from the same lot and were chosen randomly, we can only speculate either that the utilization (digestibility) of the protein (amino acids) was decreased or that compounds depressing growth were formed during storage.

FISH PROTEIN CONCENTRATE

The same methods were used to determine protein quality as were used with the freeze-dried fish.

Table 7 shows the data obtained from the feeding tests made on FPC's produced from the fish held in iced storage. All the FPC's gave a greater gain in weight and a higher pro-

TABLE 7.—Mean weight gained, food consumed, and protein efficiency ratio of groups of eight rats fed diets of FPC prepared from raw fish stored in ice for periods up to 11 days compared with casein.

Storage time	Mean weight gained	Mean weight of food consumed	Adjusted protein efficiency ratio[1]
Days	*Grams*	*Grams*	
2	154.0 ± 8.63	363 ± 12.0	3.62 ± .05
6	155.1 ± 8.12	362 ± 12.3	3.65 ± .09
8	154.4 ± 4.95	368 ± 7.6	3.59 ± .10
11	145.4 ± 4.80	358 ± 10.3	3.47 ± .10
Casein	113.5 ± 5.65	323 ± 3.9	3.00 ± .00

[1] The protein efficiency ratios were adjusted to a protein efficiency ratio of 3.00 for casein.

tein efficiency ratio than did the casein. Diets containing FPC made from fish stored for 2, 6, and 8 days in ice resulted in protein efficiency ratios ranging between 3.59 and 3.65. The diet containing FPC from the 11-day-old fish yielded a slightly lower gain in weight and a protein efficiency ratio of 3.47. These results agree with those obtained with the standard reference samples made from the same fish. The nutritive quality of the 11-day standard reference samples, however, was poorer than that of the FPC sample. This anomalous result suggests either an improved utilization of protein as a result of extraction with isopropyl alcohol or the removal of some factor that may have depressed growth.

Freeze-dried fish produced from whole red hake stored in ice 2 to 8 days did not differ in protein quality. Freeze-dried fish produced from whole red hake stored for 11 days, however, was lower in protein quality but still had a protein efficiency ratio equal to that of casein. FPC produced from whole fish stored for 2 to 11 days showed no differences in protein quality. All the FPC's had protein efficiency ratios higher than that of casein.

SUMMARY AND CONCLUSIONS

Whole red hake were stored in ice for 2, 6, 8, and 11 days. The fish were organoleptically evaluated for freshness at each storage period and were then processed by freeze-drying to form a reference sample or by solvent extraction with isopropyl alcohol to form a fish protein concentrate. These products were then analyzed for proximate composition and amino acid concentration and for protein quality.

The results of the subjective evaluation for freshness indicated that the fish stored up to 8 days were still acceptable for food but that those stored for 11 days were not acceptable.

The proximate composition and the amino acid concentration of the freeze-dried whole samples of fish showed very little change as a result of the storage of the raw fish in ice. Rat-feeding tests indicated a loss in protein quality of the freeze-dried sample prepared from 11-day-old raw fish. Protein efficiency ratio values ranged from 3.35 to 3.49 for fish stored up to 8 days, whereas the 11th-day sample resulted in a protein efficiency ratio of 3.18. All protein efficiency ratio values, however, were equal to the value for casein or were higher.

The proximate composition of FPC's produced from fish stored up to 8 days in ice remained relatively constant. The crude protein in the concentrate produced from fish stored for 11 days decreased about 2.5%. The concentration of amino acids also followed this pattern with a resultant lowering in the concentration of lysine and a slight increase in that of proline and glycine. The protein quality of the FPC processed from the 11-day-old fish was also slightly lower than that of FPC processed from fresher fish. All FPC's, however, had protein efficiency ratios higher than that of casein.

We conclude that storage of whole hake in ice up to 8 days is a satisfactory means of holding them prior to extracting the ground hake with isopropyl alcohol to produce FPC.

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LABORATORY REARING OF THE DESERT PUPFISH, *Cyprinodon macularius*

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ABSTRACT

The desert pupfish, *Cyprinodon macularius*, may be reared in the laboratory for use in the study of embryology, genetics, physiology, and behavior. It is euryhaline (0-70 ‰) and eurythermal (8°-44.6° C) and may be useful as a bioassay for either freshwater or marine pollutants. In the Salton Sea area of California, the recent introduction of exotic species and the encroachment of civilization have drastically reduced the formerly abundant pupfish populations. Laboratory rearing eliminates the need for continuous exploitation of a rapidly contracting natural population and could supply adequate stocks for sanctuaries, thereby preserving the species from extinction. Laboratory apparatus and conditions are described for maintaining larval and adult pupfish. Parasites and diseases encountered are discussed and successful treatments described. Methods for spawning and rearing the desert pupfish in the laboratory are detailed. These methods may also be applicable to many other species of pupfish that are in danger of extinction.

The desert pupfish, *Cyprinodon macularius* Baird and Girard, is a killifish (Cyprinodontidae) native to the Lower Colorado River Basin from southern Arizona to southern California and the Sonoyta River of northern Sonora, Mexico (Miller, 1948). It thrives under the harsh conditions of the desert environment. It lives in fresh water as well as highly saline pools that few other vertebrates can tolerate. Its ability to survive in such environments, plus other important biological characteristics listed in Table 1, renders it an exceptionally hardy laboratory animal potentially valuable for research in many fields.

POTENTIAL FOR RESEARCH

Desert pupfish has many characteristics favorable for embryological research. It can be spawned with relative ease and can be maintained in the laboratory throughout the year to supply large eggs (approximately 2 mm in diameter), suitable for vital marking and grafting experiments. Other favorable characteristics are the transparent chorion and the long developmental period which can be temperature-controlled (New, 1966). The sticky filaments that are attached to the chorion of its demersal egg can be partially removed by rolling the eggs gently on filter paper 4 to 8 hr after fertilization. Any remaining filaments are matted together and can be easily removed with small forceps.

Desert pupfish, since they mature quickly, could be used for research in fish genetics and on the aging process. Barlow (1961) reported that pupfish reach maturity in the field in 3 months. F_1 pupfish, reared from eggs and maintained at 27° C, were observed spawning in the laboratory approximately 4 months after hatching.

The desert pupfish, a euryplastic species, also possesses physiological and behavioral traits that make it valuable for scientific research. The juveniles can tolerate salinities ranging from fresh water to 90 ‰ (Barlow, 1958). The adults, although less euryhaline, are known to spawn in salinities as high as 70 ‰ (Kinne and Kinne, 1962). The salinity tolerance of newly hatched pupfish render them potentially useful for comparative bioassay of freshwater and marine pollution. The extreme temperature tolerance is an additional asset. Desert pupfish

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TABLE 1.—Biological characteristics of the desert pupfish, *Cyprinodon macularius*, and other important fish in research.[1]

Items	Desert pupfish, *Cyprinodon macularius*	Trout: *Salmo trutta*, *S. gairdneri*	Killifish, *Fundulus heteroclitus*	Medaka, *Oryzias latipes*
Salinity tolerance:				
Adults	0-70 ‰	Euryhaline	Marine-estuarine	Fresh water
Eggs	0-90 ‰	Fresh water	Wide tolerance	— ? —
Natural spawning season	April to October[2]	Spring or fall	June to August	April to October
Artificial spawning	Spawns naturally all year, cannot be stripped	Seasonal spawning, eggs are stripped	Seasonal spawning, eggs are stripped	Spawns naturally all year, eggs can be stripped
Egg type	Demersal; transparent	Demersal; opaque	Demersal; transparent	Demersal; transparent
Egg diameter	Ca. 2 mm	Ca. 5 mm	Ca. 2 mm	Ca. 1 mm
Incubation temperature Range	13°-36° C; at 20° C, 10 days	3°-13° C; at 10° C, 34-37 days	15°-27° C; at 20° C, 9.5 days	13°-25° C; at 25° C, 8 days
Length of adult	4-5 cm	15-30 cm at first spawning	8-12 cm	2-4 cm
Age at maturity	3-4 months	2-4 years	1-2 years (?)	1-2 months

[1] Data compiled from: Barlow, 1958, 1961; Frost and Brown, 1967; Kinne and Kinne, 1962; Miller, 1970 (personal communication); New, 1966; Rugh, 1962; Trinkaus, 1967; Yamamoto, 1967.
[2] Desert pupfish has not been recorded spawning throughout the year in nature as have other species of pupfish but presumably would do so under the proper conditions (Bunnell, 1970).

have been found in the field at temperatures ranging from 8° to 44° C (Lowe and Heath, 1969; Kinne, 1960).

Since it can be relatively easily spawned in the laboratory, the desert pupfish is a good model for the study of reproductive behavior. Numerous and rapid behavioral sequences precede the actual spawning act. On occasion, however, fish in high spawning readiness eliminate many behavioral sequences and commence spawning immediately. When properly stimulated the fish swim parallel to one another, the male slightly behind the female, twist into an S-curve, and spawn. Release of the gametes is accompanied by a quivering movement of both fish. The male wraps his anal fin under the female's vent and fertilizes each egg as it is extruded. The female then dips, leaving the fertilized egg attached to the substrate. This process is repeated until the female is spent, having spawned 50 to 200 eggs in about 2 hr under laboratory conditions. In nature the female only rarely spawns more than one or two eggs in succession (Barlow, 1961). The mature male is easily recognized by his aggressiveness and his brilliant, blue coloration. The pugnacious male pupfish must be separated in the laboratory from females and other males. The determination of the male to spawn, regardless of circumstances, makes the pupfish a potentially valuable species for classroom demonstrations. Barlow (1961) has presented a complete and detailed description of the social and reproductive behavior of the desert pupfish.

LABORATORY REARING THE DESERT PUPFISH FOR CONSERVATION

Another value in rearing desert pupfish lies in the preservation of the species. Today the pupfish faces elimination from many areas of its natural range due to predation and competition from exotic species and the modification or destruction of its habitat. Large populations of desert pupfish, once prevalent around the Salton Sea, have been alarmingly reduced. At the present rate of population reduction, the species may well become extinct in this area in the near future unless steps are taken to insure its survival. Artificial rearing is one of the possible means.

Coleman (1929) conducted an ecological survey of the Salton Sea for the California Depart-

ment of Fish and Game and judged that the numbers of mosquitofish, *Gambusia affinis*, and desert pupfish were sufficient to support a large population of carnivorous game fish. Cowles (1934) reported the pupfish populations to be exceedingly large in and around the Salton Sea. In 1956 Barlow (1961) observed schools of juvenile pupfish of nearly 10,000 individuals. He estimated that one large, isolated, shore pool at the Salton Sea contained 150 adults per square meter. Today, in the Salton Sea area desert pupfish are almost totally confined to a few tributaries. In response to the severe reduction of pupfish populations in the Salton Sea area, Jack Hesemeyer, Supervisor of the Anza-Borrego State Park, has built a pupfish sanctuary near the Park headquarters at Borrego Springs. This small sanctuary was stocked on June 24, 1970, with 48 laboratory-reared fish produced by the techniques outlined below. Several hundred additional fish were placed in the Palm Canyon pools nearby.

The authors hope that this article, in addition to demonstrating the value of the desert pupfish as a teaching and research animal, will help in its preservation by describing laboratory-spawning methods that can provide adequate stocks for sanctuaries in natural and artificial habitats. In addition, the rearing techniques described here for desert pupfish may be useful for the preservation of many other species of pupfish that are in danger of extinction.

MATERIALS AND METHODS

The desert pupfish used to develop spawning techniques were seined from an irrigation ditch emptying on the northwestern shore of the Salton Sea in Riverside County, Calif. Specimens were transported in plastic garbage pails filled with aerated ditch water to the Fishery-Oceanography Center at La Jolla, Calif. We found that the water temperature during transport should not be allowed to fluctuate radically from that at which the fish are found.

Many of the specimens collected were infected by a freshwater parasitic copepod of the family Lernaeidae (possibly introduced with home-aquarium fish). The large egg cases of this copepod were clearly visible on the fish, usually at the base of the fins. Individuals carrying this parasite were weak and commonly died during or soon after transport. Reichenbach-Klinke and Elkan (1965) recommend a salt bath (NaCl, 0.76-1.1%) to eliminate such copepods. To treat this infection, all fish on arrival at the laboratory were converted to seawater over a 5-day period. Kinne (1960) reported that a 1-month-old pupfish can survive sudden salinity changes up to 35 ‰, whereas 1-year-old adults cannot survive sudden salinity changes of more than 10 to 15 ‰. Robert R. Miller (1970, personal communication), however, reports that in 1937 he found that this species could be shifted with ease directly from fresh water to seawater and back. After conversion to seawater all traces of the parasitic copepod vanished. Toward the end of the experiment, several fish died from a devastating protozoan infection in the epithelial tissue surrounding the mouth. The tissue appeared bloody and often had completely disintegrated. Many apparently healthy fish died with little or no warning in less than 12 hr. The marine parasitic protozoan, *Cryptocaryon irritans*, prevalent in the Scripps water system was suspected (Wilkie and Gordin, 1969). The surviving fish were transferred back to fresh water and, fortunately, the parasite failed to make the transition.

LABORATORY CONDITIONS

The fish were maintained in 20-gal tanks with subsurface filters covered with crushed oyster shell. The water was changed completely and the shell washed approximately every 6 weeks. Four individuals were isolated in each aquarium by plastic, perforated dividers. The tanks were maintained at room temperature, 20° to 22° C. Standard aquarium heaters were used whenever higher temperatures were needed. No attempt was made to control pH other than the use of the crushed oyster shell substrate.

The fish were fed frozen adult brine shrimp, *Artemia salina*, three times daily during the week and once a day on weekends. Kinne (1960) and Kinne and Kinne (1962) used two species of

worms (*Enchytraeus albidus*[3] and *Tubifex* sp.), two species of crustaceans (*Daphnia* and *Cyclops*), beef liver, fresh lettuce, spinach, and several brands of commercial fish food.

The fish were subjected to a daily 16-hr light and 8-hr dark cycle. The lighting used was a combination of daylight fluorescent bulbs and mercury vapor arc lamps. Most fish were ready to spawn under these conditions within 3 weeks of capture, although there was marked seasonal and individual variation. Observations made suggest the length of the light period is more important than light intensity for the induction of spawning. Kinne and Kinne (1962) reported using a 14L-10D cycle with a combination of fluorescent tubes and natural daylight.

SPAWNING METHODS

The fish were spawned on a varied schedule, depending on the readiness of the female. The average female spawned 50 to 200 eggs, depending upon her size, approximately once a week. One large, exceptionally prolific female spawned 200 eggs twice a week for 2 months. Subsequently, this female was not spawned on schedule, became eggbound, and was unable to extrude her eggs. After her death the large, single ovary contained over 800 eggs and accounted for 44 % of the total body weight. Females that would not spawn at room temperature were spawned at 27° C. Females that could not spawn at either room temperature or 27° C were removed from isolation to a community tank (4 females, 2 males) on a 12L-12D photoperiod, wherein they quickly spawned out their eggs at 27° C. Kinne (1960) reported that the fish do not spawn in the laboratory at temperatures below 20° C and seem to spawn optimally between 28° and 32° C. He also noted that the temperature in the field varies between 25° and 35° C for most of the spawning season.

Kinne and Kinne (1962) reported that pupfish embryos have one period of low thermal stability between fertilization and gastrulation and a second period just before hatching. Observations made during our study indicate another period of low thermal stability just before fertilization. When a female maintained at room temperature is transferred to a higher temperature to induce spawning, most of the eggs either are not fertilized or do not develop. We found that females should be maintained and spawned at one temperature. If maintained at a temperature above 25° C, they will need to be spawned regularly; otherwise, the eggs are dropped to the bottom of the aquarium unfertilized. A more flexible spawning schedule is possible if the female is kept at a lower temperature.

The fish were spawned in white, plastic, food containers measuring 27 × 20 × 10 cm, containing 2.5 liters of water at 22° C. Immediately after the spawning these containers were suspended in a water bath at 27° C. This technique produced good hatches in spite of the reported low thermal stability between fertilization and gastrulation (Kinne and Kinne, 1962). The water bath was a 20-gal tank with a standard aquarium heater.

Either a green plastic mat or white cheesecloth was used as a substrate on which the females could attach the adhesive eggs. On several occasions, when the plastic mat was used, the pupfish were observed eating the previously spawned eggs, which were readily visible against the green background. The substitution of cheesecloth with L-shaped glass-rod weights at its periphery successfully reduced parental egg consumption. The cheesecloth was a superior substrate because many of the eggs were buried in the material and thus were inaccessible to the parents. Furthermore, the combination of a white spawning bin, white cheesecloth, and nearly transparent eggs made the latter virtually invisible to the experimenters and, presumably, to the fish.

The parent fish were well fed prior to spawning to help reduce the number of eggs consumed. The spawning bin, containing the female, was placed in a quiet location. Five to fifteen min later the male was introduced to the spawning bin which was then left undisturbed for 1 to 2 hr. Barlow (1961) reported that spawning

[3] See Kinne (1960) for details on mass culturing *Enchytraeus albidus*.

lasts from 30 min to 2 hr, depending on the size of the female and the number of eggs laid. In order to prevent the serious injury or death of the female, the male was not left in the spawning bin for longer than 2 hr. After termination of spawning the fish were returned to their aquaria and the feces were removed from the container. The spawning bin was then suspended in a 27° C water bath with aeration. Kinne and Kinne's (1962) data on hatching shows that any incubation temperature between 24° and 30° C should produce hatches of at least 80 % in 100 % air-saturated seawater. Pupfish eggs left at room temperature in the laboratory at La Jolla suffered extremely high mortalities owing to daily temperature fluctuations between 18° and 24° C. Kinne and Kinne (1962) supplied data on egg mortalities and incubation periods at different temperatures from 10° to 37° C.

Hatching success of different breeding pairs varied unexplainably under constant conditions. A large sample of eggs from one breeding pair showed, however, that salinity and temperature markedly effected the hatching success of pupfish eggs (Table 2). Eggs in small clusters, apparently laid at nearly the same instant, seldom hatched. Kinne and Kinne (1962) also reported reduced development and increased mortalities for conglomerated eggs.

Pupfish larvae are large enough (5.5 mm total length at 27° C in 50 % seawater) to feed from the day of hatching on brine shrimp nauplii (Salt Lake variety), *Artemia salina*. The larvae when handled were drawn with a bulb into a long glass tube. Kinne and Kinne (1962) gave extensive data on the growth, food intake, and food conversion for pupfish larvae at different temperatures and salinities.

TABLE 2.—Hatching success of desert pupfish eggs.

Salinity	Temperature	Eggs			
		Developing		Dead	Total
	° C	No.	%	No.	No.
Fresh water	22	74	54.0	66	140
	27	4	5.4	70	74
	27	3	5.0	57	60
Half seawater	27	92	85.1	16	108
Seawater	27	17	29.6	40	57
	27	13	29.5	31	44
	27	7	16.6	35	42

CONCLUSIONS

The desert pupfish may be reared in the laboratory over a wide range of temperatures and salinities. Half-strength seawater at 27° C provided the best hatch observed during this study. The pupfish is a hardy laboratory animal that does well in captivity if proper attention is paid to food, space, and hygiene. However, a note of caution should be added, since we reared only two generations of pupfish. Bunnell (1970) states that when a pupfish stock is bred in captivity from a single pair, the fish do well at first, but gradually die out over several generations. This possibility should be further substantiated before committing experimental studies to a single line of descent.

The authors believe that the desert pupfish is an excellent experimental animal for many types of biological research. Studies of the systematics (Miller, 1948), behavior (Barlow, 1961), and physiology (e.g., Kinne and Kinne, 1962) provide a wealth of background information on the basic biology of pupfish which will prove valuable to investigators interested in using this species in teaching and research. Other reports (e.g., Bunnell, 1970) indicate the critical status of some species of *Cyprinodon*, including *C. macularius*, and point out the need to provide sanctuaries to avoid extinction of this unique species. Laboratory rearing of pupfish will not only provide material for scientific observation and experimentation, but will also remove some of the pressure on an already rapidly contracting natural population by providing adequate stocks for present and future sanctuaries. Both measures should enhance the value of the desert pupfish and emphasize the importance of saving the species from extinction.

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GONAD MATURATION AND HORMONE-INDUCED SPAWNING OF THE GULF CROAKER, *Bairdiella icistia*[1]

IRWIN HAYDOCK[2]

ABSTRACT

Successful methods of capture, transport, disease treatment, and laboratory maintenance of the gulf croaker, *Bairdiella icistia*, are described. Gonad maturation was achieved out of season by the use of appropriate controlled photoperiods, temperatures, and abundant feeding. Mature fish or fish brought to maturity in the laboratory were spawned with suitable hormone injections and the time of spawning could be accurately predicted. Eggs obtained from hormone-induced spawning were normal in all respects and the larvae were reared through metamorphosis using rotifers and brine shrimp nauplii as food; thus, the life history of this marine fish can, for the first time, be completed under controlled laboratory conditions. The techniques developed for croakers may have application to mariculture, biossay of marine pollution, and in more general research on marine fish reproduction.

Reproduction in fishes which spawn annually is controlled by the interaction of exogenous and endogenous stimuli, particularly the endocrine activity of the pituitary-gonadal axis which is indirectly influenced by environmental factors such as day length and temperature. Against this basic background, which determines the gonadal maturation cycle, the actual spawning act is controlled by neurohumeral and neuromuscular connections activated by rapidly changing environmental parameters and behavioral cues (Liley, 1969; Malven, 1970). This basic reproductive pattern and the lacunae in our understanding of it have recently been reviewed in detail (Breder and Rosen, 1966; Hoar, 1969).

Much of the work on reproductive physiology of fishes has been done with freshwater species of interest to aquarists (Wickler, 1966) or experimentalists (New, 1966; Hoar, 1969; Liley, 1969). There is, however, no reason to suspect that the basic features of reproduction, elucidated with freshwater species convenient to maintain, will differ significantly from those of marine forms, which require more elaborate facilities for study.

Because of increasing demand for fish as a source of protein, as well as for sport and for scientific studies on reproductive success of fishes in generally deteriorating natural environments, there are now intensive and extensive efforts to artificially culture commercially valuable freshwater species (Hickling, 1962; Hora and Pillay, 1962; Bardach, 1968). Recent interest has also focused on mariculture, the cultivation of marine species. Some success has been achieved in Great Britain (plaice and sole—Shelbourne, 1964 and 1970), Japan (bream and yellowtail—Harada, 1970), and in the United States (pompano—Finucane, 1970). However, all such cultures are started either with young fish caught at sea or with eggs spawned in the sea or stripped from mature fish captured during their normal spawning season. The field work necessitated by this method can be costly and unpredictable, drawbacks which are compounded by the difficulty usually experienced in simultaneously finding running-ripe fish of both sexes, especially females. These problems are avoided in the culture of salmonids because of the unique determination of the fish to return to the same place to spawn at a fairly predictable time (Leitritz, 1959). The grunion is a striking

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example of a marine fish showing similar determination (Walker, 1952).

The necessity of working with the reproductive products of one species only during its normal spawning season is one of the major obstacles faced by experimentalists and fish culturists. Most species of temperate and high latitudes show rather well-defined, short spawning seasons, probably related to the annual cycle of day length, temperature, and associated productivity. This seasonality generally means that experimental work proceeds at a hectic pace for a short time and then must be dropped or switched to another species available in breeding condition. This causes numerous difficulties, delays, and expenses for both research and researcher. The answer to this problem is to artificially induce maturation and spawning under controlled laboratory conditions.

To my knowledge, artificial gonad maturation under controlled conditions of light and temperature, hormone-induced spawning, and laboratory rearing of the fragile larvae through metamorphosis have not been accomplished for any single marine fish prior to this time. Pompano have been spawned with hormones, but the fertilized eggs did not hatch (Finucane, 1970). A euryhaline form, *Fundulus heteroclitus*, has been spawned with pituitary extract (Joseph and Saksena, 1966); however, only mature fish recently obtained from their natural environment were used.

Protocols for hormone-induced spawning of numerous species of freshwater fishes is well established (Dodd, 1955; Fontenele, 1955; Clemens and Sneed, 1962). A useful review of literature on the effects of hormones in fishes (Pickford and Atz, 1957) has been updated with a comprehensive, annotated bibliography (Atz and Pickford, 1964), and a timely review of various aspects of reproductive physiology of fishes is also available (Hoar and Randall, 1969, 1970).

Hormone-induced spawning is an accepted part of several commercial fish culture ventures and will be used in many more when techniques become reliably standardized for various species. Brazilians pioneered the use of hormones in spawning carp, while in Russia, where hydroelectric dams block the spawning migrations of sturgeons and salmonids, hormone-induced spawning has been practiced for many years (Atz and Pickford, 1959). In India, carps spawn naturally in flowing streams but must be injected with conspecific pituitaries before they will spawn in ponds (Chaudhuri, 1960; Das and Khan, 1962). Catfish respond to similar treatment (Sundararaj and Goswami, 1968). In the United States, several freshwater and anadromous species are spawned with hormones (Ball and Bacon, 1954; Clemens and Sneed, 1962; Stevens, 1966).

My investigation focused on the use of hormones in inducing maturation and spawning of the gulf croaker, *Bairdiella icistia* (Jordan and Gilbert), both for the specific purpose of obtaining eggs for physiological studies of salinity tolerance and for the more general purpose of studying factors which influence spawning in marine fishes. Once success had been achieved in spawning fish under controlled laboratory conditions, the influences of biological and physical factors on the spawning process were examined in detail.

THE SALTON SEA FISHERY

The Salton Sea is a large, saline, inland lake in the lower desert of southern California. The present body of water was formed when the flood waters of the Colorado and Gila Rivers broke through irrigation dikes in 1905 and poured into the then dry Salton Sink. The irrigation canals were repaired and the water again brought under control in 1907. Subsequently, the Salton Sea was declared an agricultural sump for the deposition of large quantities of irrigation waste water. This water leached large quantities of salt out of the surrounding agricultural land and carried it into the sea. Consequently, over the years the salinity gradually increased. Short-term fluctuations occurred—reflecting changes in inflow, annual rainfall, surface area, temperature, and evaporation. The salinity of the Salton Sea in 1970 is about 37 ‰.

During the period 1950-56, when the salinity of the Salton Sea approximated that of the ocean,

the California Department of Fish and Game transplanted a variety of fishes from the Gulf of California with the intent of establishing a productive sport fishery. The present fishery stems from the descendants of a total original introduction of 57 gulf croaker, 67 sargo, *Anisotremus davidsoni*, and 100 to 200 orangemouth corvina, *Cynoscion xanthulus*, transplanted to Salton Sea in 1951-52 (Walker, 1961; Whitney, 1967). The fishes thrived and developed large populations in the simple, man-made ecosystem, but increasing salinity now threatens the continuing existence of the famous fishery.

The Salton Sea Investigations was a joint Federal-State project whose overall goal was to predict a target salinity at which a water quality control project could be aimed that would not be detrimental to the present highly esteemed fishery. Engineering studies had suggested a method by which the salinity of the Salton Sea could be controlled, but the maximum permissible salinity levels still had to be established on the basis of biological information. Other sciaenid species live in the Gulf of Mexico in a wide range of salinities up to 75 ‰, but it is considered unlikely that spawning occurs in extremely high salinities or that the larvae could tolerate such osmotic stress (Gunter, 1967; Hedgpeth, 1959).

To attain our goal in the short time available, we have developed all of the necessary techniques for spawning Salton Sea fishes under controlled laboratory conditions. These techniques have proved successful for obtaining viable spawn for the necessary salinity tolerance studies of both croaker and sargo.[3] I had originally assumed that the close relationship of gulf croaker to corvina, both of the family Sciaenidae, would make it possible to apply croaker spawning techniques directly to the corvina. However, the carnivorous nature of corvina and the general difficulty of handling this powerful fish in the laboratory demanded special feeding and holding techniques and precluded successful laboratory spawning. Hormone-induced spawning has been achieved in our laboratory with other mature marine fishes, including *Eucinostomus* sp. (family Gerridae), *Genyonemus lineatus* (Sciaenidae), and sargo (Haemulidae). The techniques developed for inducing spawning with hormones in gulf croakers may thus have general applicability.

[3] Reuben Lasker and Richard R. Tenaza. 1968. Salton Sea fish larvae investigation progress report: techniques and preliminary experiments on osmotic stress (spring-summer 1968). Inland Fisheries Administrative Report No. 68-7, Oct. 1968, (Unpublished).

METHODS

CAPTURE, TRANSPORT, CARE, AND HANDLING OF FISH

Two year classes of gulf croakers were captured with a 60-m beach seine in May and October 1969. The first sample consisted of mature fish, 1 year old, and the second of young-of-the-year fish which were subsequently matured under controlled photoperiod and temperature conditions in the laboratory. The fish were captured on sandy beaches north of the U.S. Navy Base and north of the Salton Bay Marina, both on the west side of the Salton Sea. A beach seine is the most dependable method for capturing large numbers of these fish in good condition, except in midsummer and late winter, when fish are unavailable near shore.

The fish were transported to La Jolla, Calif., in a 500-liter tank equipped with aeration and filtration devices. In transport, and for several subsequent days in the laboratory, the fish were treated with Furacin antibiotic[4] at an initial concentration of 250 mg/3.8 liter. Several early failures showed that careful handling, high standards of water quality, and, especially, antibiotic treatment are all essential to high survival rates of Salton Sea fishes after capture.

In the laboratory, the fish were held in 2,000-liter rectangular tanks (2 m × 1 m × 1 m deep) supplied continuously from the seawater supply of the Fishery-Oceanography Center in La Jolla. A general description of this extraordinary facility is available (Lasker and Vlymen, 1969). A water temperature of 22 ± 1° C was main-

[4] Sharpe and Vejar, Los Angeles, Calif. Use of trade names is merely to facilitate descriptions; no endorsement is implied.

tained except for special studies or for short maintenance periods. An artificial photoperiod of 16 hr light:8 hr dark (16L:8D) was maintained by time clocks (Tork, Model 7100[5]) in a light-tight room with 400 w, white mercury lamps (Sylvania, H33-1-GL/C[6]), suspended 1.5 m above the water surface at the center of each tank. Deviations from this water temperature and light schedule are reported where appropriate.

Young fish, less than 13 cm in length, were fed 0.238 cm Oregon Moist Pellets[8] from Allen automatic trout feeders[7] activated with time clocks (Paragon Model 4001-0[8]). As fish grew larger their consumption of pellets diminished until they were fed exclusively on squid which was ground semifrozen into pieces ranging from 0.5 to 2.5 cm^2. All fish were fed squid ad libitum twice daily during the week and once a day on weekends. I consider adequate feeding to be an important factor in gonad maturation, but it was not a variable in this study. In the laboratory, young fish doubled their weight in 2 months and, later, added about 10 g each month, reaching 80 to 100 g by the end of the first year. Salton Sea fish weigh 40 to 50 g at the end of 1 year; the largest gulf croaker caught during this study weighed 420 g.

Initially, and for later studies of the effects of a series of hormone injections, fish were never disturbed without first draining the tanks to within 10 to 15 cm of the bottom and then adding anaesthetic until the fish could be touched without causing a sudden reaction. Fish to be injected were then removed and completely anaesthetized for 1 to 3 min in MS-222 (Tricaine Methanesulfonate)[9] at 0.6 g/3.8 liters. Treated fish were replaced in fresh seawater; if necessary, fish were held with gloved hands and moved rapidly back and forth at the surface to aerate the gills and assist their recovery. There is little danger of fish mortality when the anaesthetic is properly used, and no adverse effects on fish reproduction were found. Later, experience enabled us to net unanaesthetized fish rapidly and place them directly into buckets with MS-222 until they were unconscious. This was done routinely when spawning techniques were thoroughly known.

Fish were individually marked with subcutaneous injections of a 65 mg/ml stock solution of Bismark Brown or Fast Turquoise PT.[10] Hormone, antibiotic and other injections were carried out with 0.5- to 1.0-cc disposable syringes fitted with 25- to 26-gauge, 1.27- to 1.90-cm (½-¾ inch) needles. Needles 0.95 cm (⅜ inch) long proved useful for intraperitoneal (ip) injection where internal damage was possible, but these allowed too much fluid to escape when intramuscular (im) injections were used. In most cases the needle was carefully slipped into the skin between scales, and carrier fluid (oil and saline) was slowly injected into the deep muscles of the back adjacent to the dorsal fin. Slow withdrawal and pressure over the wound site helped to retain most of the fluid. Sesame oil was used in most cases as a carrier, but no essential differences were noted between oil and Holtfreter's saline injections.

LIST OF HORMONES AND THEIR PREPARATION

1. Oxytocin (Pitocin, Pituitary grade I).[11] Injectable solution, 20 IU/ml, used as obtained and stored at 4° C.
2. Deoxycorticosterone Acetate (DOCA, grade II).[11] Injected as a slurry in sesame oil.[12]
3. Human Chorionic Gonadotropin (HCG, stock No. CG-2).[11] Anhydrous powder was made to volume with Holtfreter's saline just prior to injection.
4. Gonadotropin from Pregnant Mare's Serum (PMS).[11] Powder dissolved in distilled water and stored at —10° C.
5. Carp Pituitary (freeze-dried powder).[13]

[5] Pacific Wholesale Electric Company, San Diego, Calif.
[6] R. V. Moore Company, La Conner, Wash.
[7] G-Z Company, Sacramento, Calif.
[8] J. F. Zwiener Company, San Diego, Calif.
[9] Crescent Research Chemicals, Scottsdale, Ariz.
[10] Allied Chemical Company, San Francisco, Calif.
[11] Sigma Chemical Co., St. Louis, Mo.
[12] A. Sahadi Co., Moonachie, N.J.
[13] Stoller Fisheries, Spirit Lake, Iowa.

Powder was homogenized wt/vol in sesame oil or Holtfreter's saline.

6. King Salmon, *Oncorhynchus tshawytscha*, Pituitary.[14] Glands removed and placed in acetone for extraction within 15 min of spawning. Whole glands were homogenized wt/vol in sesame oil or Holtfreter's saline.

Multiple intramuscular (im) or intraperitoneal (ip) injections were accompanied with 200 IU Potassium Penicillin G and 0.025 mg active Streptomycin Sulfate. Fish that were handled several times, with disposable plastic gloves, were routinely treated with Furacin water-mix antibiotic (Vet. grade), at an initial concentration of 250 mg/3.8 liters.

PREPARATION OF SALMON PITUITARIES

Early in the project my attention was directed to the possible usefulness of fish pituitaries for inducing spawning (Pickford and Atz, 1957; Clemens and Sneed, 1962; Atz and Pickford, 1964). Carp pituitaries are commercially available, but there is no assurance that these are removed from spawning fish, a time at which the glands are assumed to have high titers of spawning hormones. Glands from *Bairdiella*, taken during the spawning season, would be ideal, but the bony nature of the brain case makes their removal difficult and their small size makes the effort relatively unrewarding. Spawning grunion, *Leuresthes tenuis*, are seasonally abundant locally but, again, collecting a large number of glands would require great effort. Salmon provide a convenient source of fish pituitaries, since they are available in large numbers at fixed locations (fish hatcheries). Each gland is of considerable size (about 13 mg dry weight), and the bony brain case is reduced to a soft cartilage by decalcification near the time of spawning. Each fish is graded at the hatchery so that spawning females are only sacrificed when at the peak of running ripeness. Fish return to spawn at different times at various hatcheries which allows some flexibility in the timing of collecting operations. All these advantages, plus the fact that the glands proved useful in spawning croakers, justify a detailed discussion of the method developed by Nimbus Hatchery personnel for removing the pituitary from king salmon. I thank W. H. Jochimsen and D. R. Von Allmen for originally demonstrating this technique to me.

A technique for removing pituitary glands from salmon with a special tool exists (Tsuyuki, Schmidt, and Smith, 1964), and similar equipment was available at the Nimbus Hatchery. However, hatchery personnel have developed a simple and rapid technique which allows one person, with a little practice, to directly remove 100 to 200 pituitary glands during the course of a morning's spawning activities at the hatchery. The number of fish spawned limits the number of glands obtained; several hundred fish are spawned each week during November, the peak season at Nimbus Hatchery. The largest numbers of fish enter the hatchery ponds from the river on overcast days or during winter rains.

Female and male pituitary glands were removed from spawned fish within 15 min of death (unfortunately, following death, a severing of the head artery which bleeds the fish often destroys the pituitary in the process). The fish to be used for pituitary extraction are held upright with the gill cover slipped over a sharp stake clamped to a table. A large, sharp butcher knife is used to slice through the cartilaginous tissue of the brain case, parallel to the jaw and just above the level of the eye (a metal glove, as worn by fish-market personnel, would be useful for this step, which is carried out while the neuromuscular system of the large fish is still active). A rubber coat and rubber boots are also necessary accouterments at this gross stage of dissection. The cut exposes the brain, or, if at the appropriate depth, the pituitary stalk (sometimes the gland itself) is severed and the gland can be removed from its cavity with a pair of forceps or a narrow scoop. Some cuts, at odd angles, sever nerve cords as well as the pituitary stalk and expose three cavities. Once learned, the location, consistency, and color of the pituitary differentiate it from nervous or other fatty tissues with which it might be confused. At first, shallow cuts can be made to

[14] Nimbus Fish Hatchery, Rancho Cordova, Calif.

expose the brain; the gland's cavity and stalk are readily seen when the brain is lifted from its cavity and the entire gland can be removed with forceps. A direct approach is more appropriate to the speed with which spawning activities proceed and 1 day of practice is sufficient to enable one person to remove glands as fast as fish are available without undue loss of damaged or partial pituitaries.

The glands are placed directly in chilled acetone until spawning and gland-taking activities are finished. The acetone is decanted and replaced 2 or 3 times over a period of 3 days until all loose material is washed away. The final wash of acetone is decanted, and the glands are blotted gently on filter paper, placed in tightly stoppered vials, and kept at —10° C in a jar of desiccant. Glands stored 1 or 2 years by this method showed no detectable loss of activity.

It was found that pituitaries were easier to homogenize if they were not completely dried. Just prior to preparing a stock solution, the glands were removed from vials, air-dried for a few minutes, and weighed. They were then immediately placed in glass tissue grinders and homogenized in sesame oil or saline. The resulting brei was finely divided and was used unfiltered without clogging 25- and 26-gauge needles. Stock solutions (1-10 mg ml) were stored in rubber-capped serum bottles (5 ml) at —10° C. All injections were 1.0 to 0.5 ml im and up to 1 ml ip. I used oil rather than saline in most cases on the assumption that oil may slow the rate of absorption and more evenly distribute the hormone.

EVALUATING THE EFFECTS OF HORMONE TREATMENT

Three basic criteria—gonad index, spawning, and fertilization of eggs—were chosen to test the effects of gonadotropin injections. Development of fertilized eggs to hatching is also a useful test of the absolute success of the hormone treatment used to obtain spawn, but hatching success is also very sensitive to other factors, e.g., salinity, temperature, dissolved oxygen, and bacterial contamination.

A commonly used measure of the effects of hormone treatment is the gonosomatic index (GSI) which expresses wet gonad weight as a percent of total wet body weight. The GSI is a reasonable measure of the state of reproductive maturity of a fish. Its measurement, of course, requires that the fish be sacrificed. Histological examination of gonads or measuring egg diameters to assess the stage of maturation are more elaborate approaches which were not used after it was found that GSI accurately predicted spawning readiness of the fish. McInerney and Evans (1970) have shown a direct relationship between GSI and the histological index in female threespine stickleback. Among more recent approaches to this problem is Stevens' (1966) development of a technique for removing eggs from striped bass by means of a catheter. He was able to determine the stage of the eggs and predict the time of optimal ripeness with a fair degree of accuracy.

Samples of 4 or 5 fish were used to assess the effects of hormone treatment. Controls of uninjected or sham-injected groups of fish were maintained where these were appropriate to understanding the effects of hormone treatments on GSI. The GSI of laboratory-held fish varied, understandably, through time, and the effects which this had on the results obtained are discussed where appropriate. The dates of most experiments are given to help explain this variation.

Two fundamental processes in spawning, hydration and ovulation, were evaluated separately with respect to various hormone treatments. Hydration was measured as an increase in total body weight over a period of 1 to 2 days after injection, a process distinct from gonad growth which occurs over longer time periods. This rapid weight gain is due mostly to water uptake by the fish and is reflected in much higher GSI values, as most of the water appears to go into the gonad. Externally, a hydrated fish is grossly bloated, and, in some cases, the fish are listless and remain motionless on the bottom of the tank. Ovulation was assessed by attempting to strip eggs from fish at various times after injection. Eggs may be forced from nearly ripe fish, but these eggs invariably are surrounded

by a single layer of vascularized ovarian tissue. Overripe eggs have no covering but are spotted with areas of coagulated yolk. Usually, eggs taken within 1 to 2 hr of ovulation are perfectly round, 0.7 to 0.8 mm in diameter, and have the single oil drop characteristic of this species. These eggs float in normal Salton Sea water (37‰) and are perfectly transparent and viable.

Fertilization of viable eggs and development to hatching was followed for random samples of eggs obtained from hormone treatments. Eggs which did not cleave were considered unfertilized. In most cases, test fertilizations were conducted in charcoal-filtered Salton Sea water, which has an ionic composition different from that of normal seawater (Carpelan, 1961). Fertilization and hatching served as final criteria of the usefulness of various preparations for spawning.

EXPERIMENTAL RESULTS

The reproductive process in fishes can be experimentally divided into four phases: (1) gonad maturation—measured as a slow increase in GSI over several weeks; (2) gonad hydration —the final preparation of reproductive products for spawning; (3) the actual spawning act—releasing eggs and sperm; and (4) fertilization and development of the eggs. These phases are interdependent and proceed in a timed sequence as part of a cycle keyed to external and internal cues which are integrated by the fish. Thus, spawning is the culmination of a whole series of events, each of which has particular physical and biological requirements for successful completion.

Although each process in the spawning cycle can be separately evaluated with appropriate experiments, it is important to keep in mind that the results must be judged against the general reproductive status of the organism. For example, the effects of hormone injections differ among croakers with different GSI's. For reasons explained in the following section, most of the experiments were focused on the reproductive processes of female croakers.

GONAD MATURATION (MALES)

Effects of Photoperiod and Temperature

Male croakers became running ripe in the laboratory under all combinations of experimental conditions. Fish of 20 to 30 g, captured in October, well beyond the normal breeding season of the species in Salton Sea, became running ripe within 3 weeks after being placed in tanks with various combinations of 14° C and 22° C water and 16L:8D or 8L:16D photoperiod schedules. Adult fish, captured at the height of the breeding season in May and June, were still ripe under all laboratory conditions in November and remained in this state until the end of the experiments in July of the following year. In the field, males become running ripe at least 1 month prior to the natural spawning season and can thus serve as indicators of the approaching breeding season in Salton Sea. They are not ripe prior to this time or after about 1 month following the spawning season, although water temperatures and day lengths are similar to those maintained in some laboratory situations in which the males did remain running ripe. It may be that either the absence of the normal Salton Sea cycle of light and temperature conditions (in which the fish experience very warm water—25°-30° C—followed by a period of winter dormancy), or the omission of the normal spawning act in the laboratory, helped to maintain the fish in running ripe condition. The threshold at which environmental factors induce spermiation may also be quite low, and in the laboratory, food, light intensity, photoperiod, and temperature acting in concert may have exceeded this level.

Effects of Hormones

Hormone injection of 1 mg salmon pituitary caused a seminal thinning response similar to that discussed by Clemens and Grant (1964). In comparison, the milt taken from uninjected fish was quite viscous and formed clots which had to be mechanically broken up to disperse the sperm. However, quantitative injections of smaller doses of salmon pituitary caused no apparent increase in the percent water of the testes (Table 1). In males, the reaction to hormones

TABLE 1.—Total weight, GSI (gonad weight/body weight × 100) and percent water in testis of male *Bairdiella icistia* injected with various dosages of salmon pituitary.

Treatment	Wet weight of fish (mean ± SD)	GSI (mean ± SD)	% water in gonad (mean ± SD)	N
Control: 0.01 ml/g sesame oil	67.96 ± 13.38	7.03 ± 1.13	85.2 ± 1.08	5
Salmon pituitary 0.005 mg/g	59.97 ± 11.67	5.25 ± 1.61	86.0 ± 1.20	5
Salmon pituitary 0.01 mg/g	59.34 ± 6.99	5.53 ± 1.74	85.8 ± 1.14	5

was not comparable to hydration in females and was not further pursued. Possibly stronger doses of pituitary would give a positive response. The time scale and extent of the seminal hydration and thinning response might be useful in assessing the effect of various hormones and dosage relationships where female fish are at a premium and males would otherwise be in excess. This bioassay technique has been used for salmon pituitary gonadotropin (Yamazaki and Donaldson, 1968a, 1968b).

GONAD MATURATION (FEMALES)

Seasonal Maturation Cycle in the Salton Sea

A series of croaker samples was taken from the Salton Sea at monthly or more frequent intervals during the year. GSI values were less than 2 % from January to mid-March 1969, prior to spawning, and less than 1 % from mid-June to December 1969, following spawning. From mid-March to mid-April, the mean GSI increased rapidly from 2 % to 10 % and reached a high of 12 % in mid-May (Fig. 1); at this time, individual females were caught with GSI's of more than 17 %. Peak spawning in the Salton Sea was observed in May and early June in 1969. Less frequent sampling and observations confirmed a similar pattern of events in 1970. In the years 1955, 1956, and 1957, Whitney (1961) found that the peak abundance of croaker eggs and larvae in the Salton Sea fell in middle and late May.

Laboratory Cycle and Effects of Photoperiod, Water Temperature, and Food

Immature fish.—Young-of-the-year croakers were captured in October 1969, placed in 14° C or 22° C water on a 16L or 8L photoperiod, and given an abundance of food (Oregon moist chow and chopped squid). These fish grew to maturity in a little less than 4 months at 16L:8D and 22° C (Table 2, group 1). Similar fish kept on 8L:16D at 14° C did not grow as rapidly as warm-water fish and did not mature under these conditions (compare groups 1 and 2, Table 2, 16-II-70). The lack of maturity was not due to the slower growth rate of the fish kept in cold water, as Salton Sea fish of lower average weight showed higher GSI values at their capture during the spawning season (Table 2, group 3). Short days, therefore, inhibited the maturation process. These same fish (Table 2, group 2) did mature rapidly, in 2 months, when the water temperature was increased to 22° C along with increased photoperiod. Young fish in their first year do not have as high a percentage of gonad as fish older than 1 year (Table 2, groups 3 and 4).

Mature fish.—A sample of fish more than 1 year old, captured in November 1968, 6 months

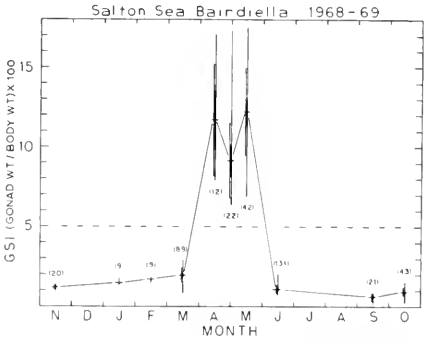

FIGURE 1.—Seasonal change in GSI of female *Bairdiella icistia* captured in the Salton Sea. Horizontal line indicates the mean GSI value; vertical line indicates range; on either side of the mean, open bar indicates the standard deviation, and closed bar two standard errors of the mean of each sample. The number of fish sampled is given in parentheses. Horizontal dashed line indicates 5 % GSI for comparison with laboratory fish (Fig. 2).

TABLE 2.—GSI values of female *Bairdiella icistia* captured in Salton Sea compared with captured fish matured in the laboratory.

Group	Date of sample	Weight (mean ± SD)	GSI (mean ± SD)	N
1	23- X -69[1]	10.47 ± 3.56	0.96 ± 0.28	9
	5-XII-69	34.30 ± 5.15	0.93 ± 0.15	11
	19-XII-69	35.40 ± 4.78	1.07 ± 0.46	10
	16- II -70	60.14 ± 11.84	5.46 ± 3.32	5
2	23- X -69[1]	10.47 ± 3.56	0.96 ± 0.28	9
	5-XII-69	31.90 ± 8.04	0.92 ± 0.14	10
	19-XII-69	33.10 ± 4.94	0.80 ± 0.09	9
	16- II -70	39.40 ± 7.16	1.28 ± 0.22	5
	4- IV -70	49.30 ± 5.01	9.69 ± 3.46	5
3	14- V-70[1]	32.38 ± 8.32	5.90 ± 3.84	25
4	14- V-70[1]	170.4 ± 84.1	7.93 ± 1.35	24
5	19-III-69	161.6 ± 22.8	10.2 ± 1.94	23

Treatments

Group 1: Laboratory stock fish sampled irregularly. All fish kept on 16L:8D photoperiod at 22° C.

Group 2: Laboratory stock seporated from group 1 and mointained on 8L:16D photoperiod at 14° C until 16-II-70, when they were switched step-wise (15 min/day) to 16L:8D and 22° C.

Group 3: Young fish captured during their first breeding seoson in Solton Seo.

Group 4: Fish more than 1 year old, captured during the breeding season in Soltan Seo.

Group 5: Fish more than 1 year old, captured fallawing the breeding season in Saltan Sea and matured early under laboratory conditions of 15L:9D and 14° to 16° C.

[1] At capture.

before their normal breeding season, reached maturity in the laboratory sometime prior to being sampled in mid-March 1969 (Table 2, group 5). These fish experienced 15L:9D and ambient La Jolla seawater temperature (14°-16° C) during 4 months in the laboratory. Cold water may slow down the maturation process, but it is evidently not as important as the stimulation of long days.

A second sample of adult fish was captured in mid-May (at the peak of the breeding season), subjected to various experimental laboratory conditions and sampled every 2 weeks to determine the status of their GSI (Fig. 2). Fish maintained on long days (16L:8D) at 14° C and 22° C showed a slow decline in GSI from a high of 12 % at capture to below 5 % by late-August and September. The GSI values at 22° C were more variable and, in general, showed a more rapid decline than those at 14° C. Similar fish given 10L:14D at high and low temperatures showed a similar but much more rapid decline in GSI, and their GSI also declined to a lower overall level (1-2 % by mid-August) than that of fish which never experienced short-day conditions.

Both groups of short-day fish subsequently showed a slow but steady increase in their GSI in response to having the photoperiod increased 15 min/day from 10L to 16L. Although this increase was not followed through a complete cycle, it was evident that exposure to long days for 2 to 3 months would have been required to bring the fish up to the GSI level necessary for spawning (about 5 %—see below).

It is likely that adult fish brought into the laboratory just prior to the normal increase in GSI observed in the Salton Sea would respond rather quickly, I estimate within 1 month, if they were given adequate light, temperature, and food. It may also be possible to mature fish rapidly after they have gone through their natural GSI decrease (Fig. 1), but this was not tested.

Effects of Hormones on Maturation of Fish Maintained in the Laboratory

Groups of fish maintained on various light and temperature regimes were subjected to hormonal treatments to enhance gonad maturation. Adult fish captured prior to the breeding season were not available for these experiments, which were conducted after the spawning season on fish undergoing a decline in GSI, as in Fig. 2. Nonetheless, the results obtained probably indicate the extent to which maturation can be influenced by hormone treatment. In croakers, the technique of hormone-induced maturation is of relatively little practical importance, since fish can be matured by appropriate manipulation of photoperiod, temperature, and feeding schedules. The results are, therefore, reported for their possible application to other species in which maturation proves more intractable.

Since the treatments were carried out on fish being used for photoperiod and temperature experiments, the results can only be evaluated in relation to the GSI value of the population under each set of conditions. In some cases, sham-injected controls were used while in others uninjected fish sampled for the light-temperature

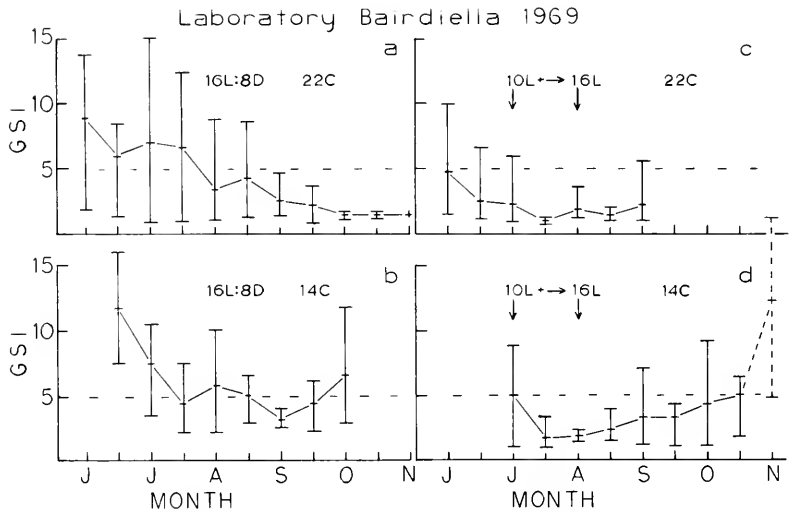

FIGURE 2.—GSI of female *Bairdiella icistia* captured during the spawning season and maintained under the following laboratory conditions: (a) 16L:8D, 22° C; (b) 16L:8D, 14° C; (c) 10L:14D, 22° C for 2 months; then, photoperiod was increased 15 min/day to 16L:8D; (d) 10L:14D, 14° C for 2 months; then, photoperiod was increased 15 min/day to 16L:8D. Mean GSI of all fish at capture (May 13, 1969) was 12.2 %. Dashed value in (d) indicates GSI of five fish injected with 1 mg of salmon pituitary 1 day prior to last sampling period. Horizontal line at 5 % GSI indicates approximate minimum level necessary for successful hormone-induced spawning.

studies served as controls when they were scheduled to be sampled at the same time as the injected fish. In all cases the gonad index responded positively to hormone treatment. Differences seen in the tabular data (Tables 3, 4, and 5) were due to the number of injections, strength and type of hormone injected, the water temperature and the GSI value of the fish at the beginning of the experiment.

In Tables 3, 4, and 5 the symbols (S, E, +, —) record the initial (2 days after the first injection) and the maximal (at some point during the series of injections) response noted for each group of fish. Two factors, water temperature and GSI, were found to have an important bearing on the results observed following hormone injection. Fish kept in 14° C water showed the most consistent and largest positive response to long-term hormone injections which were giv-

TABLE 3.—GSI of *Bairdiella icistia* given five injections, every other day for 9 days (17-26-IX-1969). Photoperiod was 16L:8D and temperature 14° C during this experiment. The response of the fish to injection was assessed every day and the maximal response is indicated by S (= spawned viable eggs), E (= obtained nonviable eggs), + (= swollen papillae observed, no eggs obtained), or — (= no observed response). The second column under each heading indicates the reproductive status of the fish 2 days after the first injection. The third column (in parentheses) indicates the maximum reaction noted during the experiment.

Treatment	1 mg salmon pituitary extract	100 IU HCG	5 mg DOCA	Uninjected control
GSI	12.4 + (E)	12.1 — (E)	11.4 — (E)	4.1 (—)
	11.6 + (E)	10.0 — (E)	6.1 — (E)	3.6 (—)
	11.1 + (E)	8.8 — (+)	5.2 — (E)	3.2 (—)
	10.9 — (—)	6.6 — (+)	4.6 — (+)	2.7 (—)
	6.0 — (+)	5.9 — (—)	male	2.7 (—)
$\bar{X} \pm SD$	10.4 ± 2.6	8.7 ± 2.5	6.8 ± 2.7	3.2 ± 0.6

TABLE 4.—GSI of *Bairdiella icistia* given six injections over a 15-day period (18-VIII-69 — 2-IX-69). Each injection consisted of 1 mg salmon pituitary extract. Fish in groups 1 and 2 experienced a 16L:8D photoperiod; groups 3 and 4 experienced 10L:14D for 2 months (to 28-VII-69) followed by day length increases to 16L:8D (by 22-VIII-69). Groups 1 and 3 were maintained at 22° C, groups 2 and 4 at 14° C throughout the experiments. Symbols (S, E, +, —) are the same as in Table 3.

	Group 1 (16L, 22° C)		Group 2 (16L, 14° C)		Group 3 (10L-16L, 22° C)		Group 4 (10L-16L, 14° C)	
	Injected	Control	Injected	Control	Injected	Control	Injected	Control
	6.5 E (E)	10.2 (—)	11.3 + (E)	10.2 (—)	5.6 — (E)	3.6 (—)	7.3 — (E)	2.4 (—)
	5.0 E (E)	3.2 (—)	9.4 + (E)	6.0 (—)	4.9 — (E)	1.6 (—)	3.3 — (+)	2.0 (—)
	4.2 + (E)	1.9 (—)	9.1 + (E)	5.5 (—)	1.8 — (+)	1.4 (—)	1.9 — (—)	1.9 (—)
	4.2 + (+)	1.3 (—)	9.1 + (E)	5.2 (—)	1.6 — (—)	1.4 (—)	1.7 — (—)	1.6 (—)
	3.6 — (—)	0.9 (—)	7.6 + (E)	2.3 (—)	1.2 — (—)	1.3 (—)	1.2 — (—)	1.4 (—)
$\bar{X} \pm SD$	4.7 ± 1.1	3.5 ± 3.8	9.4 ± 1.3	5.8 ± 2.8	3.2 ± 2.0	1.9 ± 1.0	3.1 ± 2.5	1.9 ± 0.4

TABLE 5.—GSI of *Bairdiella icistia* given three injections, one every other day for 7 days (16-IX—23-IX-1969). Photoperiod was 16L:8D and temperature 22° C during this experiment. Symbols (S, E, +, —) are the same as in Table 3.

1 mg salmon sesome oil	1 mg salmon Holtfreter's	1 mg carp sesome oil	0.1 mg salmon sesome oil	Control sesome oil
5.5 S (S)	5.0 S (S)	8.1 + (E)	6.0 — (E)	4.9 (—)
4.9 S (S)	5.0 S (S)	6.3 + (E)	3.3 — (+)	1.4 (—)
4.7 — (E)	4.6 — (E)	2.7 — (E)	3.0 — (+)	1.4 (—)
4.1 — (E)	3.6 — (E)	2.7 — (+)	2.5 — (+)	1.1 (—)
1.8 — (—)	1.4 — (—)	2.5 — (+)	1.5 — (—)	1.0 (—)
$\bar{X} \pm SD$ 4.2 ± 1.4	3.9 ± 1.5	4.5 ± 2.6	3.2 ± 1.7	2.0 ± 1.6

en every other day for 1 to 2 weeks (Table 3; Table 4, groups 2 and 4). Fish in 22° C water also showed a positive response (Table 4, groups 1 and 3; Table 5); however, this response is less clear because warm-water fish occasionally shed their eggs prior to sampling and this reduced the observed GSI.

In general, fish with GSI values below 5 % did not respond to the first injection (—), responded with a weak swelling in the genital area (+), or gave nonviable eggs (E) only after several injections. This indicated that threshold values of GSI and temperature exist below which "growth" and above which hydration and ovulation occur in response to hormone injections. These values will be further discussed in the section on ovulation. Here it will suffice to point out that hormone treatment does give rise to increases in gonad size which can perhaps be considered the equivalent of gonad growth.

The tabular data indicate that salmon pituitary caused the greatest increase in GSI of fish kept in cold water. Salmon was followed by HCG and then DOCA (Table 3). Salmon pituitary produced smaller increases in warm water than in cold (Tables 3, 4, and 5), and the response tended to vary in proportion to the dosage used (Table 5). The relatively small response at 22° C in Table 5 was probably due in part to the lesser number of injections (3) in this batch and in part to the fact that three of the fish spawned relatively large quantities of eggs (see qualitative responses in Table 5), thus reducing their GSI values, which were measured after testing for the presence of viable eggs. However, the results of the longest series of injections (Table 4) suggest that there is a general pattern of greater increase in GSI in cold water, except in the case of fish with a very low initial GSI.

In warm water (Table 4, group 1; Table 5), fish produced viable eggs (S) or nonviable eggs (E) which could be forced out the day after the first injection. Cold-water fish (Table 3; Table 4, groups 2 and 4) required several injections to produce a response and never spawned viable eggs on stripping.

In a further experiment, young-of-the-year fish collected in October were injected shortly thereafter with various concentrations of salmon pituitary to assess usefulness of immatures as a bioassay in testing dose-response relationships. Each fish received five injections over a 10-day period. At each dose level a large number of fish (20) was injected, but, because of the difficulty in identifying the sex of these immature fish, the number of females actually injected was somewhat smaller. Also, the gonads were quite small, and the overall GSI response was slight. This made any meaningful analysis difficult. How-

TABLE 6.—GSI of young female *Bairdiella icistia* after five injections of various concentrations of salmon pituitary extract over a 10-day experimental period (4-XI-14-XI-69).

Dose	Weight (g) mean ± SD	GSI mean ± SD	N
Control sesame oil	26.07 ± 5.78	0.79 ± 0.08	13
0.1 mg salmon	23.90 ± 6.23	0.82 ± 0.22	10
0.5 mg salmon	28.40 ± 4.46	0.88 ± 0.18	13
1.0 mg salmon	26.97 ± 6.88	0.99 ± 0.19	17

ever, the results (Table 6) do indicate a general increase in GSI corresponding to dose. That these fish were not too small to respond to treatment is indicated by the high GSI observed in similar sized fish captured in Salton Sea during the breeding season (Table 2, group 3). It is possible that the rather small response observed was due in part to the fact that the fish were handled frequently and did not feed readily during the course of the experiment. This test would probably be more successful if carried out with 30- to 50-g fish kept under short-day conditions at 14° C.

GONAD HYDRATION

Water Uptake Following Hormone Injections

Weight gain on various hormones.—Short term changes in body weight occurred 1 to 2 days following the injection of various hormones. This weight change was recorded as a percent of the initial total body weight (Table 7) and was found to vary from slightly negative values to positive values of over 13 % of the body weight. Single injections were given, and fish were weighed prior to, and 30 hr after, injection. With few exceptions, the fish showed little or

TABLE 7.—Effects of hormones of GSI of *Bairdiella icistia* at 30 hr post-injection. (1 = did, 0 = did not hydrate or ovulate.)

Preparation, dose, and date	Initial body weight (g)	Weight change (% initial weight)	GSI (% final weight)	Hydrate	Ovulate
Carp pituitary 10 mg					
10-VI-70	51.07	+10.99	9.34	1	0
	70.46	+5.42	19.80	1	0
	72.79	+11.72	25.50	1	0
	57.22	+12.82	28.60	1	0
Carp pituitary 5 mg					
16-VI-70	71.40	--	5.42	0	0
	54.22	--	8.14	0	0
Salmon pituitary 0.1 mg, 5-VI-70	48.33	−0.93	2.88	0	0
	55.90	−2.45	3.60	0	0
	49.40	+2.39	10.97	(1)	0
	66.14	+0.06	12.41	0	0
Salmon pituitary 0.5 mg, 2-VI-70	65.18	−2.59	3.83	0	0
	62.77	−1.96	5.02	0	0
	74.83	−0.64	5.18	0	0
	56.22	+8.75	21.76	1	1
Salmon pituitary 1 mg					
28-IV-70	96.12	+9.40	15.90	1	1
15-V-70	65.50	+13.40	--	1	1
18-V-70	79.90	+13.10	--	1	1
15-V-70	131.20	+6.00	--	1	1
Salmon pituitary 2 mg					
10-IV-70	93.30	--	24.47	1	1
23-III-70	68.92	--	25.67	1	1
10-IV-70	100.03	--	28.08	1	1
Salmon pituitary 5 mg					
28-V-70	66.1	+7.35	20.79	1	0 (few eggs)
	65.4	+6.11	21.23	1	0 (few eggs)
Salmon pituitary 5 mg—Can.	91.7	+10.09	25.94	1	1 (poor eggs)
	62.3	+8.06	26.64	1	0 (few eggs)
PMS 100 IU					
27-IV-70	86.8	--	21.00	0 (?)	1
	--	--	--	0 (?)	1
12-V-70	88.7	+4.18	--	1	1
7-V-70	173.6	+11.98	24.02	1	0
PMS 50 IU					
9-VI-70	80.38	−3.06	2.90	0	0
	54.89	+4.10	14.60	1	1
	69.90	+7.77	18.50	1	1
	62.18	+12.06	24.10	1	1
HCG 100 IU					
28-IV-70	68.5	--	28.76	1	0
14-IV-70	87.5	--	29.37	1	0
15-IV-70	84.5	--	30.29	1	0
HCG 50 IU					
16-VI-70	78.51	−2.09	2.69	0	0
	57.03	−1.95	3.65	0	0
	67.91	−1.29	3.68	0	0
	80.41	+7.15	19.80	1	1
Oxytocin 20 IU					
18-VI-70	59.12	−0.70	1.60	0	0
	65.66	−1.02	3.37	0	0
	72.11	−0.11	3.95	0	0
	60.08	+2.39	11.97	1	0
DOCA 5 mg					
15-VI-70	54.78	−0.51	4.07	0	0
	53.70	−3.07	4.28	0	0
	55.33	−0.55	7.98	0	0
	73.10	−1.20	9.78	0	0

no response to 0.1 and 0.5 mg salmon pituitary, 5 mg carp pituitary, 50 IU HCG, 20 IU oxytocin, and 5 mg DOCA. On the other hand, 1 to 5 mg salmon, 10 mg carp, and 100 IU HCG, gave uniformly positive results, all fish showing weight gains of 5 to 13 %. Variable results were observed with 50 and 100 IU PMS. Although most PMS fish which were weighed showed some gain in weight, this was in general less than that observed with carp, salmon, and HCG. In fact, it was noted that two fish injected with 100 IU PMS spawned freely without ever appearing grossly bloated, a characteristic of all fish which were spawned with other preparations.

The time scale of weight gain.—A comparison was made of the weight gained by fish given one injection of 5 mg salmon, 10 mg carp, and 50 IU PMS (Table 8). The time span of hydration was arbitrarily divided into the weight gained between 7 and 23 hr post-injection and the total weight gained, including that added between 23 and 30 hr. At 30 hr ovulated eggs, if present, were stripped from the fish. Generally, all fish lost weight in the first 7 hr, probably because of handling and lack of feeding during the experiment. The weight gains are due mostly to water uptake and movement of water into the gonad.

TABLE 8.—Effects of hormones on time-course of weight gain.

Preparation and dose	Initial fish weight	Percent weight gain	
		7-23 hr post-injection	7-30 hr post-injection
	G	%	%
Salmon pituitary 5 mg	66.1	4.58	7.35
	91.7	7.72	10.09
	65.4	4.59	6.11
	62.3	4.41	8.46
Carp pituitary 10 mg	57.22	7.38	13.13
	51.07	4.17	10.44
	72.79	6.76	13.11
PMS 50 IU	54.89	0.13	5.25
	62.18	4.86	13.11
	69.9	2.88	9.69

The results show that the weight increase in the final 7 hr prior to spawning is less than 50 % of the total increase with salmon pituitary, more than 50 % with PMS, and about equal when carp pituitary is used. This evidently reflects some fundamental difference in the way these preparations affect the physiological mechanism causing hydration. The time-course of hydration (Table 8) may be important in determining the condition of eggs at ovulation (Table 7). It should be noted that among the three groups tested for the time-course of hydration, viable eggs were obtained only from the PMS-injected fish (Table 7); unfortunately, there is no comparable data on the time-course of weight gain in fish given 1 mg salmon, which also produces viable eggs.

Factors Affecting Hydration

GSI.—It is apparent from Table 7 that the gonad must be close to 5 % of the body weight to respond to an otherwise adequate dose of hormone. Although GSI could not be measured prior to injection, almost all fish which failed to respond had final GSI's below 5 %. Table 9 presents further confirmation of this. These fish, injected with 1 mg salmon, came from a stock which had shown a general decline in GSI, because of being kept on a long photoperiod for an extended time. Of four injected fish, three hydrated and one of these subsequently spawned. The fish that neither hydrated nor spawned had a final GSI of just under 3 %. Only 1 of 11 uninjected fish from this same stock showed a GSI above 5 %, while 3 more were above 4 %.

TABLE 9.—GSI of *Bairdiella icistia* measured on 26-VI-70.

A. Fish injected with 1 mg salmon pituitary, after GSI had declined (1 = did, 0 = did not hydrate or ovulate).

Initial body weight	Weight change (% initial weight)	GSI (% final weight)	Hydrate	Ovulate
53.10	+14.73	24 42	1	1
65.38	+7.40	10.55	1	0
60.14	+4 21	6.94	1	0
79.89	−2.21	2.77	0	0

B. Uninjected fish from some tank.

Total weight	GSI
67.65	1.63
53.20	1.79
93.28	0.05
58.86	4.38
63.48	1.27
61.90	4.57
78.60	1.70
74.70	2.08
53.24	2.59
69.29	7.86
59.46	4.80

Hormone dosage.—In general, there appears to be a threshold response to dosage. Fish with high GSI values and between 50- and 100-g body weight hydrated after one injection of 10 mg carp but not 5 mg (Table 7). With salmon, 1 to 5 mg were adequate doses for 50- to 350-g fish while 0.1 and 0.5 mg were inadequate except in one case. In the case of HCG, 100 IU caused hydration while 50 IU did not except in a single case. Note, however, the low GSI value measured for three of the fish given 50 IU HCG.

It must be noted that the highest dosages used were adequate for hydration but inhibited spawning (see section following on ovulation). This was true for 10 mg carp, 5 mg salmon and, especially, 100 IU HCG where the fish continued to gain weight and eventually died in the tank without ovulating a single egg. These results would suggest that it is important to determine the lowest possible dosage which will consistently bring about hydration.

Temperature.—A temperature threshold underlies the entire spawning process. Between 14° and 17° C the fish did not hydrate in response to an otherwise adequate dosage of 1 mg salmon pituitary. Two days later these same fish spawned within 30 hr when given a second 1-mg-salmon injection 24 hr after being transferred to 22° C water.

OVULATION

Ovulation and Hydration as Separate Events

Some early results with 100-IU-PMS and 100-IU-HCG injections led to speculation that the two hormones were acting on different physiological processes. PMS brought about ovulation without gross hydration while HCG hydrated fish to the point of death without ovulation (Table 7). This result was not confirmed with 50-IU doses, but, in general, the impression gained was that PMS produced high quality eggs with less hydration than either HCG or salmon pituitary.

In sharp contrast to the PMS results, HCG caused uniform hydration but, with one exception, failed to bring about ovulation. In a preliminary test, it was found that oxytocin (20 IU) or salmon (1 mg) caused some eggs to be ovulated when the injection was given 24 hr after the fish were injected with HCG. Oxytocin and salmon pituitary had the same effect on carp-injected fish which otherwise did not ovulate.

Ovulation without apparent hydration was also achieved by using multiple, subthreshold doses of 0.1 and 0.5 mg salmon pituitary, but the time of ovulation could not be accurately predicted, and therefore the eggs obtained usually were not viable. (The importance of obtaining eggs just at ovulation is discussed in the section on fertilization.)

In summary, carp pituitary, HCG, DOCA, and oxytocin were uniformly inadequate for bringing on ovulation except in the case of one fish treated with HCG. Both carp- and HCG-injected fish hydrated, some becoming grossly distorted. On the other hand, salmon pituitary and PMS regularly brought about ovulation. Dosage appeared to be critical in the case of salmon pituitary, as nonviable eggs resulted from injections of 5 mg and no spawn could be obtained with a single dose of 0.1 and 0.5 mg. A 1-mg-salmon dosage seems to be optimal for fish of 50- to 100-g total weight. Both 50- and 100-IU dosages of PMS gave good results with remarkably clear eggs obtained from all fish. In one case, a 50-IU dose of HCG was adequate for spawning, but 100 IU appeared to be inhibitory to ovulation.

The Time Scale of Ovulation

The combined results from 28 fish which produced viable eggs following injection with a single dose of salmon (1-3 mg) showed that the average time elapsed from injection to spawning was 30.4 hr, with a standard deviation of 3.3 hr and a range of 24.5 to 35.5 hr (Fig. 3). This 30-hr latent period following injection generally held regardless of the type of hormone used, its dosage, or the time of day the injection was made. In the case of a few large fish (100 to 300 g) given 1 mg salmon, a second 1-mg injection was given 24 hr later, but this did not affect the time of spawning. Five fish given 5 mg salmon spawned 28 hr after injection, but their eggs were not viable. In one experiment,

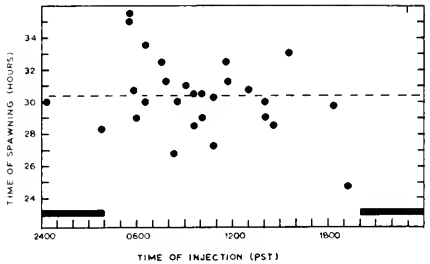

FIGURE 3.—Time of injection (Pacific Standard Time) and time of spawning (hours post-injection) in relation to photoperiod. All injections were 1-3 mg salmon pituitary extract and all fish produced viable eggs. Solid black bar indicates dark period; dashed line indicates mean time of spawning.

five fish were injected with 1 mg salmon at a time corresponding with the beginning of the laboratory dark cycle. All these fish spawned 24 hr later, indicating a possible enhancement by the normal diurnal cycle of glandular activity. Natural spawning in the Salton Sea is related to the normal diurnal light cycle, with most spawning occurring in the early evening.

Factors Affecting Ovulation

It has already been shown that GSI level, hormone dosage and type of hormone are critical interacting factors which must be considered in any spawning effort. Injection of high levels of salmon (5 mg) may possibly assure a more uniform hydration response (see GSI of Table 7), but the nonviable eggs which result speak against using more than the minimal dose found to give consistent results.

The effect of temperature on ovulation per se was not studied. Hydration is effectively blocked at temperatures lower than 17° C, but this effect was reversed after 24 hr acclimation at 22° C. In the cases in which this transfer was carried out, a second injection was given 24 hr after transfer, and spawning took place approximately 30 hr later.

As a matter of practical interest, it was found that fish could be injected and spawned twice (tried, successfully, with two fish) or three times (one fish) with a period between spawnings of 3 to 4 weeks. This is in contrast to the much longer time required for maturation after fish had slowly resorbed their gonads in photoperiod experiments (Fig. 2). Apparently the rapid emptying of the gonad consequent upon hormonal injection quickly leads to a renewed cycle of egg maturation.

The direct and indirect effects of various injections on the gonad were assessed by biopsy following spawning or the lack of spawning. These qualitative observations are listed in Table 10; no attempt is made to interpret these results, except to point out that fish injected with salmon pituitary extract had gonads most closely resembling those of naturally spawning fish.

TABLE 10.—Appearance of mature *Bairdiella icistia* ovaries during natural spawning and 30 hr after various hormone injections, and color reaction of fish to injections.

1. Salton Sea fish at spawning
   Gonad color white, light yellow or red-orange. Consistency of mature gonad is granular with patches of transparent eggs which are close to being ovulated or are lying free in the ovarian lumen.
2. Salmon pituitary extract
   Gonad color and consistency very close to naturally spawning fish. Most eggs ovulated and free in lumen. Fish blanch on injection.
3. Carp pituitary extract
   Gonad color red-orange; few eggs ovulated. In fish given 5 mg dose, blood clots appeared to be blocking oviducts near vent. Fish blanch on injection.
4. PMS
   Gonad whitish, translucent, strikingly different from other preparations. Most eggs ovulated and free in lumen. Possibly, greater degree of ovulation with less hydration makes gonad appear lighter in color. Fish do not blanch on injection.
5. HCG
   Eggs either not ovulated or partially ovulated, those not ovulated appear as white patches in the ovary. Many vacuoles and dispersed oil drops appear in eggs. Fish do not blanch on injection.
6. Oxytocin
   Ovary was very bloody. Eggs white (not hydrated); different sized eggs (mostly large) apparent in ovarian folds. Fish do not blanch on injection.
7. DOCA
   Fish showed no observable reaction.

FERTILIZATION

Relationship of Egg Viability to the Time of Ovulation

Shortly before ovulation, eggs could be squeezed from females by applying strong pressure to the abdomen, but eggs obtained in this way still had an investiture of blood vessels and ovarian tissue and could not be fertilized. An analysis of viability in relation to the time after

TABLE 11.—Fertilization and viability of *Bairdiella icistia* eggs tested over a 4-hr period following first ovulation. Fish was injected with 1 mg salmon pituitary extract at 0930 1-V-70; first eggs expressed with some difficulty at 1530 2-V-70.

Time of fertilization	Fertilization	Development to early tailbud	Hatching
	%	%	%
1530 hr (not quite running ripe)	100	91	84
1630 hr (running ripe)	100	91	83
1730 hr	100	78	72
1830 hr	90	62	44
1930 hr (eggs spotty, opaque)	5	52	34

ovulation was made in the case of one fish which remained ripe for 4 hr (Table 11). To check for viability, eggs were test-fertilized at hourly intervals following the first sign of ovulation, taken as the earliest time when normal eggs could easily be expressed from the fish by gentle pressure applied to the abdomen. Fertilization and early cleavage remained above 90 % up to 3 hr post-ovulation. By 4 hr the eggs looked crinkled, opaque, and spotty, and less than 5 % could be fertilized. A further measure of viability was made by culturing 100 early cleaving eggs from each batch until hatching. A decrease in hatching success was noted in the eggs obtained 2 hr after the initial ovulation, and hatching decreased still further in the 3- and 4-hr post-ovulatory samples. It appears that the maximum grace period for egg-taking is about 2 hr. In another experiment I studied eggs from a larger sample of 10 fish determining fertilization success as a function of time after ovulation; the optimum time for taking eggs was 1 hr after the fish first showed signs of running ripeness and gave viable eggs.

Although eggs rapidly deteriorated when kept in the ovary following hormone-induced ovulation, it was found that they retained their ability to be fertilized up to several hours after they were placed in a moist storage chamber. Eggs placed in seawater remained fertile for several minutes; in one case, a few cleaving eggs resulted from fertilization carried out after the eggs had been in seawater for 30 min.

Viability of Sperm

Although eggs kept in seawater remained viable for several minutes, sperm were no longer able to fertilize eggs 30 sec after the sperm mass had been introduced into seawater. It is thus readily apparent that croaker sperm and eggs should be mixed immediately after the sperm is obtained, in order to achieve maximum fertilization. Microscopic examination showed that sperm were immediately activated by addition of water and retained motility for a period of 1-5 min. In some tests it was apparent that water from the Salton Sea caused greater activity for a longer time than water taken from the ocean at La Jolla, Calif., but there was great variability between males, and a proper technique of quantifying this relationship awaits further studies.

Number of Eggs Obtained by Hormone Treatment

The number of eggs obtained by hormone injection varied between 700 and 1,000 per gram of fish wet weight (Table 12). This provided 50,000 to 100,000 eggs for experiments from each fish of 50 to 100 g used in this study.

TABLE 12.—Number of eggs obtained from hormone-induced spawning of *Bairdiella icistia*.

Wet weight		GSI	Ripe eggs		Actual count (1 g eggs)	Eggs/g fish weight	Approximate total eggs/ fish
Total fish	gonad		Body weight	Gonad weight			
G	*G*		%	%			
82.0	17.7	21.6	16.0	74.0	4,700	841	69,000
86.6	17.6	20.3	15.5	78.1	--	[1]793	[1]69,000
91.7	26.0	25.9	19.7	76.1	5,590	1,101	101,000
342.7	62.1	--	--	75.0	--	[1]699	[1]240,000
441.5	129.7	--	17.1	--	--	[1]878	[1]388,000

[1] Indicates values calculated from measured parameter and mean number of eggs per gram counted.

DISCUSSION

MATURATION

Many experiments have shown that gonad maturation can result from hormone therapy (for reviews see Pickford and Atz, 1957; Ahsan and Hoar, 1963; Atz and Pickford, 1964; and Hoar, 1969), but in most cases this is a long and tedious approach and has proved of practical use only on a short-term basis for elucidating mechanisms of hormone action. A recently described catheter implant technique (Frogner and Hendrickson, 1970), allowing frequent or continuous administration of hormones, has been used with partial success to mature mullet, *Mugil cephalus*, with a minimum of damage from excessive handling (Shehadeh, personal communication). A mass of tangled catheters is envisioned if this technique were applied to commercial fish production, but the ease of this method may have considerable merit for experimental situations. Implanted pituitary glands might also be used to enhance maturation, and this could easily be tested in croakers.

In the present study, a slight increase in GSI, possibly reflecting enhanced gonad "growth," followed 1 to 2 weeks of hormone injections given every other day to fish held in 22° C water. Even greater "growth" enhancement was observed in 14° C water, and these fish could have been spawned using techniques which were fully developed later in the study. However, for practical purposes, it proved simpler to mature croakers in large groups using appropriate schedules of long days, warm water, and abundant feeding.

The fact that fish kept in cold water respond to hormones by gonad enlargement without subsequent hydration or ovulation indicates that different temperature thresholds exist for these various processes. It is possible that the rate of absorption of hormone is considerably slowed in cold water, as some fish do develop a slight reaction following several days of injection at 14° C.

The general relationship of light and temperature to gonad maturation is well known (see Harrington, 1959; Henderson, 1963; Wiebe, 1968; and Hoar, 1969, for reviews) and requires no lengthy discussion here. It is sufficient to note that long-day photoperiod (16L:8D) and high temperature water (22° C) induce gonad maturation in croakers several months prior to the normal breeding season observed in the Salton Sea. Also, a combination of long days and low temperature (14° C) will retard the normal GSI decline when the fish have been captured at the peak of breeding. This technique may prove useful for maintaining fish in a mature state for prolonged periods; such fish may be subsequently spawned following transfer to warm water (22° C) for a period of 1 day.

Studies on the relationship between maturity and spawning indicate the existence of a GSI threshold value of about 5 %, below which hormone injections are ineffective. Also, fish brought to maturity with photoperiod and temperature control eventually resorb gonadal tissues if they are not subsequently spawned. This resorption process requires several weeks, and the gonad will not grow in response to photoperiod and temperature during this time. Fish which are spawned with hormones do not show a refractory state and can be respawned within a few weeks. The practical implication of these findings is that the GSI of maturing fish should be frequently checked so that spawning can begin soon after the 5 % GSI threshold is reached and the fish should be spawned before they reach the maximum GSI value and begin gonad resorption. A useful approach would be to hold stock supplies of fish on short days at low temperature and mature separate groups as needed for experiments.

Samples of croakers taken throughout the year from Salton Sea showed that maturation is quite rapid, the GSI increasing from 2 to 10 % in a little over 1 month. It is probable that the increased light, temperature, and food stimulation available in the laboratory could bring about even more rapid maturation, but the proper fish (early spring) to test this were not obtained during this study.

HYDRATION

Hormone-induced gonadal hydration is a relatively rapid phenomenon which is completed

in a little over 1 day following injection under laboratory conditions. In croakers, the total water uptake is reflected primarily in increased gonad weight and may amount to more than a 10 % increase in total body weight. A detailed study of the gonadal hydration of carp, *Cyprinus carpio*, and goldfish, *Carassius auratus*, following injections of carp pituitary extracts showed a similar pattern of water movement into the gonad with respect to time (Clemens and Grant, 1964). These authors measured the increased water content of the gonad following injection and found that, in the case of males, the peak of seminal fluidity was 24 hr after a single ip injection. A similar response was observed with im injection. Goldfish females injected with 10 mg/g carp pituitary extract showed similar responses, increasing gonad water by up to 7.2 % over carrier-injected controls. Unfortunately, the changes they describe are in the relative water content of gonads and various other tissues including blood, and no mention is made of any increase in total body weight resulting from water taken up from the external medium.

Hydration under laboratory conditions results in a grossly distorted appearance in females, the abdominal cavity becoming bloated several hours prior to spawning. In the Salton Sea, females appear plump but never grossly enlarged at spawning. It is possible that naturally spawning females hydrate and ovulate frequently but in small amounts over the course of the breeding season and that the laboratory fish show the maximum hydration and ovulatory response because of unnatural overstimulation with the injection of salmon pituitary. Use of 10 mg carp pituitary and 100 IU HCG caused an equally strong hydration response, but generally this did not culminate in ovulation when these preparations were used alone. A dose threshold for response was indicated by the inability of 1 to 5 mg of carp pituitary to cause hydration. With 100 IU HCG, continued hydration without ovulation evidently overstressed the fish and led to their eventual death. On the contrary, PMS gave somewhat variable results, but appeared to have less effect on gonad hydration while, at the same time, proving to be a potent ovulating agent. Subthreshold doses of salmon pituitary do not appear to cause hydration, but a sequence of injections given at daily intervals will eventually lead to ovulation of small quantities of eggs. This may reflect the response of exceptionally ripe eggs which are able to hydrate and ovulate.

Thresholds of GSI (above 5 %), water temperature (between 17° and 22° C), and hormone dose (e.g., 1 mg or more of salmon pituitary for 50- to 100-g fish) exist, and if any one of these factors is below its threshold, hydration does not occur.

OVULATION

Ovulation in croakers is a rapid process, taking 1 to 2 hr for completion when induced with hormones in the laboratory. The period between injection and spawning includes the hydration phase and culminates in ovulation at about 30 hr post-injection. Stevens (1966) found a similar 30-hr latent period for fully mature striped bass, *Roccus saxatilis*. Clemens and Sneed (1962) found a shorter latent period of 15 hr for goldfish. Fontenele (1955) gave injections to several Brazilian fish species at 6-hr intervals. He stated that spawning usually occurred just prior to the 5th injection (i.e., close to 30 hr after the first injection), although in most cases the fish were allowed to spawn naturally in ponds and were not tested by stripping. Indian carp are also allowed to spawn naturally in ponds after injection. Chaudhuri (1960) states that spawning may come 6 to 8 hr after the first injection of very mature fish; if a second injection is necessary, the total elapsed time may be 14 to 18 hr. It would appear that in most recorded cases (see above and Pickford and Atz, 1957, Table 46) hormone injection will bring about final maturation and spawning within 1 to 2 days if the gonads are fully mature. In only a few cases (e.g., Joseph and Saksena, 1966) have longer series of injections been successfully used to produce viable eggs.

A constant time period for spawning latency was found to hold for croakers used in this study. When GSI, water temperature, and hor-

mone dosage all exceed certain threshold levels, the fish spawned viable eggs an average of 30 hr after the first injection. Hormone dosages above threshold and the type of preparation had no apparent effect on this result, which exhibited only small variability. Subthreshold doses of salmon pituitary did delay ovulation, but this delay could not be accurately predicted, and therefore the spawn obtained was never viable. Subthreshold doses might theoretically be useful if females were to spawn naturally in captivity, but croakers never exhibited complete spawning behavior in tanks following hormonal injections. Injection of oxytocin in hydrated females and in males appeared to cause heightened prespawning behavior, with males following and touching the vent of females, but actual spawning was not observed. Hydrated females eventually expelled their eggs into the tanks if they were not hand-stripped shortly after ovulation. Actually, the relatively constant latency and the fact that fish must be hand-stripped are highly advantageous to scheduling laboratory operations.

At Salton Sea, eggs of gulf croakers sampled from the plankton and staged at various times during the day and night showed that there is a diurnal pattern of spawning, most early cleavage stages appearing in the early evening (Whitney, 1961). The same diurnal pattern was found in a closely related species from the East Coast, *B. chrysura* (Kuntz, 1914). When the effect of this diurnal pattern on laboratory spawning was tested by injecting fish at a time corresponding to the beginning of the laboratory dark cycle, all five injected fish spawned just at "dusk," 24 hr after injection rather than the usual 30 hr. However, such enhancement was not found in any subsequent spawning attempts carried out at many different times of day and night. Clemens and Sneed (1962) found no change in latency in goldfish, groups of which were injected at 2-hr intervals over a period of 12 hr. Evidently, the injection of hormones usually overrides any effect of diurnal spawning patterns.

Clemens and Sneed (1962) found that the latent period decreased with increasing temperature, doubling from 12 hr at 30° C to 25 hr at 10° C. Croakers spawn above 20° C in the Salton Sea, and eggs develop to hatching between 20° and 30° C in the laboratory (Robert C. May, Scripps Institution of Oceanography, personal communication). However, all laboratory spawning was accomplished at 20° to 22° C, and no tests were run to determine if higher temperatures would decrease the latent period. At temperatures below 17° C, the croaker does not hydrate or ovulate in response to hormone injection.

For 50- to 100-g croakers, with GSI levels above 4 to 5 %, a single injection of 50 IU PMS, 50 IU HCG, or 1 mg salmon pituitary proved adequate to induce spawning. A dosage of 100 IU PMS and 2-3 mg salmon also produced viable eggs, but 5 mg salmon produced nonviable eggs in the four fish tested. A single injection of 10 mg (not 5 mg) carp pituitary or 100 IU HCG was adequate for hydration but not for ovulation.

An injection of 20 IU oxytocin or 5 mg DOCA apparently had little or no effect on hydration, although DOCA may have caused some slight change in the GSI.

Oxytocin may affect spawning directly. Liley (1969) reviews evidence that the spawning reflex is controlled by behavioral stimulation of the CNS which releases oxytocin. Oxytocin is used up during the reproductive season of fishes, e.g., *Fundulus* and *Oncorhynchus* (Perks, 1969).

The possibility that a second hormone, acting (in concert or independently) directly on ovulation, was absent from HCG or in too low a concentration in carp pituitary was evaluated in a preliminary way by injecting 10 mg carp or 100 IU HCG fish with 20 IU oxytocin at 30 hr or an otherwise inadequate dose (0.1 mg) of salmon 24 hr after the initial injection. Evidence was obtained for ovulation shortly after injection (oxytocin) or at 30 hr (salmon), although the eggs usually were not viable. These experiences indicated that hydration alone was not sufficient to initiate ovulation and that the latter may be a separately controlled process.

The apparent contrast observed with respect to the different abilities of HCG and PMS to hydrate and ovulate fish may possibly be

interpreted as additional evidence that a two-hormone system exists for reproductive control in fishes similar to the FSH-LH system of birds and mammals (see Ahsan and Hoar, 1963; Hyder, 1970, for details). HCG is LH-like while PMS is FSH-like; fish pituitary extracts show strong LH and slight FSH activity in mammals,, but there is a great deal of conflicting evidence and interpretation (Sundararaj and Goswami, 1966; Hoar, 1969). Other evidence indicates that PMS acts like a combination of FSH and LH when tested in mammals (Ball, 1960), but this effect can be modified by the dosage used. Hoar (1969) presently considers it likely that teleost pituitaries contain only a single gonadotropin.

Sundararaj and Goswami (1966) demonstrate how wide the range of conflicting results can be, when they report that hypophysectomized catfish, *Heteropneustes fossilis*, spawned ripe eggs after injection with appropriate concentrations of LH, HCG, PMS, and DOCA, while FSH brought about ripening but no spawning (LH contamination was possible). PMS did cause ovulation in striped bass (Stevens, 1966). HCG has been used successfully in other fish spawning studies (e.g., Sneed and Clemens, 1959; Stevens, 1966). The fact that both PMS and HCG can lead to successful spawning and, yet, reflect basically antagonistic systems in mammals should make these hormones prime targets in future experiments.

It is quite evident that considerable work remains to be done to untangle the connections between hydration and ovulation, which are certainly related events, but may be controlled by different hormones acting at different threshold levels.

The puzzling fact that one out of four 50 IU HCG fish hydrated and spawned while three out of three 100 IU fish hydrated but never spawned, might be explained by postulating that a "critical dose" exists, with doses above or below this level being unable to induce the complete sequence of spawning events. The three 50 IU fish which did not hydrate would perhaps have spawned if their GSI had been above 5 %. A "critical dose" phenomenon might also be involved in the observed difference in hydration and the complete lack of spawning obtained with 5 mg and 10 mg carp pituitary, as both groups showed GSI values above 5 %; in this case the "critical dose" might lie between 5 mg and 10 mg.

Carp is considered a universal donor by Clemens and Sneed (1962) and was successfully used to spawn several species of freshwater fishes. Bioassay with goldfish showed 100 IU HCG to be equivalent to 0.5 mg acetone-dried carp pituitary (Sneed and Clemens, 1959), and ovulation was obtained with 100-1600 IU HCG and 0.5-3.0 mg carp. Most other workers also report no inhibition of spawning from very large doses, but they all point out the critical nature of exceeding some lower threshold dose to initiate ovulation. The strength of pituitary extracts for spawning is assumed to be related to the reproductive status of the donors, a datum not given by the company selling the carp pituitaries used in the present experiments. Salmon pituitaries, however, were removed only from fish graded at the hatchery for optimal ripeness and the glands were taken within 15 min of death. Nonetheless, from the results of this study 1 mg of salmon pituitary appears to be 5 to 10 times more potent than 1 mg carp and about equal to 50 IU HCG or PMS, although definite qualitative differences in response exist. A truly valid comparison of the strength of various fish pituitary preparations can of course be made only by standardized bioassay (for reviews of methods see Clemens and Sneed, 1962; Das and Kahn, 1962; and Yamazaki and Donaldson, 1968a and 1968b).

It is clear that the effects of hormones vary with the GSI level of the experimental fish. Most of these spawning experiments were carried out over a 2-month period beginning in mid-April and ending in mid-June, while the GSI was gradually decreasing in the stock of fish used for the experiments. Thus, it is difficult to directly compare the effects of 50 IU and 100 IU HCG, as they were tested almost 2 months apart and the average GSI values of the experimental fish may have been somewhat different. The effect of the population's declining GSI is clear in the

case of a standard 1 mg dose of salmon pituitary, which caused hydration, but produced eggs from only one fish in the last test (late June 1970) carried out with the same stock of fish which had been spawned regularly with the same dose over the prior 2 months.

FERTILIZATION

Several early attempts to fertilize gulf croaker eggs all ended in failure. These eggs were obtained from hormone-induced spawning, and they appeared normal in all respects; however, the sperm mass was dispersed in the water some time prior to adding the eggs. Later studies showed that no fertilization resulted when the sperm and eggs were mixed more than 30 sec after sperm had been placed in water, while eggs retained their ability to be fertilized for several minutes when kept in water and for several hours when stored in moist chambers. The early failures to fertilize eggs thus resulted from not utilizing diluted sperm quickly enough. It is well known that sperm may be stored for long periods of time if it is maintained in concentrated form or is not activated by the diluent. The rapid decrease in the viability of sperm in water is probably important for maintaining the genetic integrity of the spawners; its significance for practical laboratory work is that sperm should be added to the eggs and not vice versa.

Sperm tended to be more active and to remain motile longer in water from the Salton Sea than in ordinary seawater from La Jolla, Calif. Moreover, developing eggs always floated in Salton Sea water (salinity in 1970, about 37 ‰ on the basis of total dissolved solids), while they sank in La Jolla seawater (33.5 ‰). These observations may have important implications for the salinity tolerance and adaptability of Salton Sea fishes (transplanted originally from the Gulf of California), matters of crucial interest in the initiation of this study of fish reproductive physiology.

Several batches of eggs obtained from hormone-induced spawning were allowed to develop to hatching, and a few of the resulting larvae were reared to metamorphosis in the laboratory on a diet of rotifiers, *Branchionus plicatilis*, followed by brine shrimp, *Artemia salina*, nauplii. Thus the entire life history of the gulf croaker can probably be completed under controlled laboratory conditions. This opens up the possibility of using this species for many other biological studies where large numbers (50,000-100,000) of pelagic eggs are desired from a marine species of known genetic history. Some of these studies are now in progress (Robert C. May, Scripps Institution of Oceanography, personal communication). It is hoped that future studies will include comparative work on this species, especially with respect to the possible adaptations of Salton Sea croakers since their separation from the Gulf of California population.

SUMMARY

1. Adult and immature gulf croakers captured by beach seining in the Salton Sea were transported to the Fishery-Oceanography Center laboratory in La Jolla, Calif., and used in laboratory studies on gonad maturation and hormone-induced spawning.

2. A bacterial disease which invariably developed on recently captured or frequently handled fish was successfully treated with Furacin antibiotic.

3. Long photoperiods (16 hr of light per 24 hr) and warm water (22° C), along with optimal feeding, accelerated the gonadal maturation of females captured prior to their natural cycle of gonadal maturation. These fish were ready to spawn in the laboratory 1 to 3 months prior to the spawning season observed in the Salton Sea. Male fish became ripe under all combinations of laboratory conditions and remained ripe throughout the study.

4. Concomitant field studies confirmed earlier work showing that female croakers ripened in April, while day length was increasing, and spawned when the water temperature reached about 20° C; peak spawning occurred in May of 1969 and 1970.

5. Mature fish, captured during the spawning season at the Salton Sea, quickly resorbed their gonads when held under short photoperiods (10 hr of light) in the laboratory, but similar fish

maintained on long photoperiods (16 hr of light) remained in spawning condition for 2 months (at 22° C) or 3 months (at 14° C) beyond the normal season.

6. At 14° C, injection of mature fish with salmon pituitary, carp pituitary, chorionic gonadotropin from human pregnancy urine (HCG), and deoxycorticosterone acetate (DOCA) caused increases in gonad size over sham-injected or uninjected fish.

7. A single injection of 1 mg (acetone dried) salmon pituitary, 50 IU of gonadotropin from pregnant mare serum (PMS) or 50 IU of HCG induced spawning in mature croakers (50-100 g) with gonad index values about 5 %. Fish with gonad index values below about 5 % did not respond to otherwise adequate hormone doses. Hormone-spawned fish could be spawned a second or third time at 1- to 2-month intervals.

8. Eggs could be stripped from the fish an average of 30.4 hr following injection. This latent period consisted of a slow hydration phase of water uptake followed by a rapid ovulation phase which released eggs from the follicles into the ovarian lumen.

9. The eggs remained viable only for 1 to 2 hr following ovulation, unless they were stripped from the fish and stored in moist chambers. Each female produced 700 to 1000 eggs per gram of wet body weight.

10. Sperm are viable for less than 30 sec after dispersion in water.

11. Low dosages (0.1 mg) of salmon pituitary were insufficient to cause hydration, while very high dosages (5 mg) caused hydration but, evidently, inhibited ovulation. High dosages (100 IU) of HCG caused fish to overhydrate and eventually die without having ovulated.

12. Carp pituitary caused hydration but was inadequate for ovulation. Deoxycorticosterone acetate and oxytocin, given alone, had little or no effect on the fish.

13. Fish did not respond to single hormone injections if the water temperature was at or below 17° C. One day of acclimation to a higher temperature was sufficient to prepare fish from cold water for spawning.

14. A few larvae hatched from eggs obtained by hormone-induced spawning were reared through metamorphosis; thus, the entire life cycle of the gulf croaker can be completed under laboratory conditions.

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HARMONIC FUNCTIONS FOR SEA-SURFACE TEMPERATURES AND SALINITIES, KOKO HEAD, OAHU, 1956-69, AND SEA-SURFACE TEMPERATURES, CHRISTMAS ISLAND, 1954-69

GUNTHER R. SECKEL[1] AND MARIAN Y. Y. YONG[2]

ABSTRACT

Harmonic functions have been fitted to time-series, sea-surface temperatures and salinities in order to facilitate studies of the oceanographic climate near Hawaii and Christmas Island. The manner in which Fourier analysis has been adapted to this application has been described. The standard errors of estimate for Koko Head temperatures and salinities are less than 0.26° C and less than 0.05‰, respectively. The standard errors of estimate for Christmas Island temperatures are approximately 60 % above those for the Koko Head temperature. The expected values of the Koko Head temperature and salinity functions have an uncertainty of ±0.1° C and ±0.015‰, respectively, when samples are obtained twice weekly. Error terms of the Christmas Island temperatures, with daily sampling, are on average 0.07° C. Harmonic analysis spanning the entire sampling duration shows that long-term variations in the Christmas Island temperature and Koko Head salinity are larger than the seasonal variations. Seasonal variations in the Koko Head temperatures are dominant and longer term variations small. The results of the harmonic analyses are presented in the appendixes: (1) a listing of coefficients that define the Koko Head temperature and salinity functions for each year and the Christmas Island temperature functions for each quarter of each year, (2) graphs of the fitted curves together with the observed values for each year.

In this paper harmonic functions are presented of sea-surface temperatures and salinities that have been regularly measured near Koko Head, Oahu (lat. 21°16′ N., long. 157°41′ W.) since 1956 and at Christmas Island (lat. 1°51′ N., long. 157°23′ W.) since 1954 (Fig. 1).

Sea-surface temperatures and salinities change in response to, and therefore reflect, sea-air interaction processes (heat exchange, evaporation minus precipitation) and oceanographic processes (advection, diffusion). For example, the mean sea-surface temperature for a month at Koko Head provides a measure of the mean heat content of the water near the surface. Thus, if the mean temperature for March is above that for February, then meteorological and oceanographic processes must have taken place to raise the mean heat content of

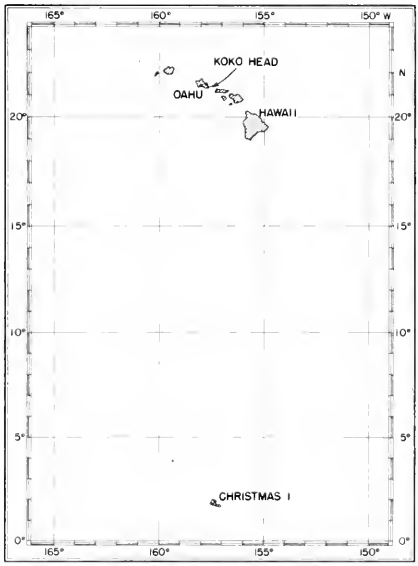

FIGURE 1.—Location of Koko Head, Oahu and Christmas Island.

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the surface water in March above that in February. This concept was used in studies of the Hawaiian oceanographic climate (Seckel, 1962, 1969) and has been applied to Hawaiian fishery problems (Seckel and Waldron, 1960; Seckel, 1963).

Rigorously, the theory of distribution of properties in the sea states that the change of sea-surface temperature during a time interval, say from the first day of one month to the first day of the next month, is equal to the integral of all meteorological and oceanographic processes affecting the temperature during the time interval:

$$\theta_b - \theta_a = \int_a^b (\text{all processes})\, dt.$$

θ_a is the temperature at the beginning and θ_b is the temperature at the end of the interval. In application, the choice of θ_a and θ_b presents the following problems: The difference in the observed temperatures at times a and b also reflects the effect of short-term variability ("noise") that is not of interest in monitoring the large-scale events. If one uses monthly mean temperatures in the heat budget equation that include observations made 15 days before and after times a and b, then the change of temperature incorporates the effect of processes that lie outside the interval of interest. Although mean values usually provide an adequate measure of the temperature change during given time intervals, the true change of temperature can be obscured. One can overcome the problems caused by the two unsatisfactory methods of obtaining measures of the temperature change by finding suitable functions that filter out undesirable short-term variability without obscuring the basic temperature and salinity trends.

Techniques that can be used in the smoothing of time series data have been reviewed by Holloway (1958) and usually involve moving averages of the data to which weighting factors have been assigned.

Curve fitting provides another method of approach. A useful technique that has been used in this report, is to obtain an analytic expression for the temperature and salinity as a function of time by Fourier analysis. The Fourier series is efficiently, and therefore inexpensively, derived by computer. Efficiency is furthered in that graphs can be produced by automatic plotter. The Fourier series provides a least-squares fit of the observed values. It permits filtering of undesired variability, facilitates statistical evaluation of the data, and—within limits—provides insight into the properties of the distribution.

These advantages will become apparent in the following sections of this report. The results of the analyses for each year of observation are presented in the appendix in both tabular and graphical form.

THE FOURIER METHOD

Fourier series are well known, widely applied, and adequately described in texts of advanced calculus. A good description can be found in Sokolnikoff (1939) where the derivation of the Fourier coefficients by least-squares method is also presented.

The temperature or salinity is expressed as a function of time, t, in the Fourier series:

$$S_n(t) = \frac{A_0}{2} + \sum_n (A_n \cos n\omega t + B_n \sin n\omega t),$$

$$n = 1,2,3, \ldots k$$

where $\omega = \frac{2\pi}{T}$, and T is the fundamental period. For example, if harmonic analysis is to be performed on data collected for a duration of 1 year, T would be 365 days.

The Fourier series contains the coefficients A_0, A_n, and B_n that are given by the Fourier integrals

$$A_n = \frac{2}{T} \int_0^{t=T} F(t)\cos(n\omega t)dt, \quad n = 0,1,2, \ldots k,$$

and

$$B_n = \frac{2}{T} \int_0^{t=T} F(t)\sin(n\omega t)dt, \quad n = 1,2,3, \ldots k.$$

The coefficient A_0 is the special case of A_n with $n = 0$. In our application $F(t)$ is the temperature or salinity at the time t. Of course, the functional relationship between temperature and time or salinity and time is not known so that

$F(t)$ is the observed temperature or salinity at the time t. Furthermore, $F(t)$ is known only at finite intervals of time so that the above Fourier integrals must be obtained by numerical integration. This integration, approximating the area under the curves $F(t)\cos(n\omega t)$ and $F(t)\sin(n\omega t)$, is performed by summing areas of rectangles with height $G(t)\cos(n\omega t)$ or $G(t)\sin(n\omega t)$, and with width Δt, the sampling interval.

The finite difference form of the Fourier integrals is

$$A_n = \frac{2}{T}\sum_{i=1}^{m} G(t)_i \cos(n\omega t)\Delta t_i, \quad n = 0,1,2, \ldots k,$$

and

$$B_n = \frac{2}{T}\sum_{i=1}^{m} G(t)_i \sin(n\omega t)\Delta t_i, \quad n = 1,2,3, \ldots k.$$

The number of samples in the interval $t = 0$ to $t = T$ is $m + 1$,

$$\Delta t_i = t_i - t_{i-1},$$

and $$G(t)_i = \tfrac{1}{2}[F(t_i) + F(t_{i-1})], \quad i = 1,2,3, \ldots m.$$

The time used to evaluate the geometric factor is $\frac{1}{2}(t_i + t_{i-1})$. Other schemes of obtaining the best estimate of $G(t)\cos(n\omega t)$ during the interval Δt can be used but would not significantly affect the results in our application (see Kaplan, 1953: p. 168-172).

Library programs for the evaluation of Fourier coefficients by computer usually require that the sampling interval, Δt, be constant. Since this condition is not necessarily met in our application, a more flexible computer program was written to evaluate the coefficients. In this program the sampling interval may vary, and the number of samples for the basic period of analysis need not be the same in each application.

The Fourier coefficients evaluated in the above manner enable us to describe analytically the temperature or salinity as a function of time. If we wish to go further and gain insight into the properties of the temperature or salinity distribution, it is more useful to express the Fourier series as a sum of cosines:

$$S_n(t) = \frac{A_0}{2} + \sum_n C_n \cos\,\omega(nt - \alpha_n), \qquad n = 1,2,3, \ldots k.$$

The transformation is accomplished by the use of the trigonometric indentities

$$A_n = C_n \cos\,\omega\alpha_n,$$

$$B_n = C_n \sin\,\omega\alpha_n,$$

$$C_n = \pm(A_n^2 + B_n^2)^{1/2},$$

and $$\omega\alpha_n = \arctan\frac{B_n}{A_n}.$$

In the application described in this report the fundamental period in the Fourier series is the sampling duration or any portion of this duration that may be arbitrarily chosen; the amplitudes and phase angles do not necessarily coincide with natural variations in temperature or salinity; and the harmonic functions have no predictive value.

In some cases, such as the Koko Head temperatures with a well-defined annual cycle, the fundamental period of the Fourier series derived for each year approximates the annual cycle. At Christmas Island, however, an annual temperature cycle is not always clearly apparent. Despite the fact that choice of the fundamental period may be arbitrary and may not coincide with a naturally occurring period, the spectrum is resolved beyond the first few harmonics. For example, if the fundamental period, $n = 1$, is 12 months then the period of the first harmonic, $n = 2$, is 6 months. A naturally occurring 9 months cycle in the observations would in this case not be resolved. As n increases, however, resolution improves to 4, 3, 2.4, 2, etc., months.

The highest harmonic, or n-value, to which harmonic analysis can be carried, is limited by the number of observations. In the ideal case and when samples are equally spaced in time, there must be at least $2n$ observations, i.e., at least two samples per cycle. In nature, where we are dealing with noncyclical variations and unequal spacing of samples a sinusoidal curve cannot be resolved with only two samples, and

a minimum of four or, better, six samples is required to achieve good resolution. For example, sea-surface temperatures are to be monitored and the fundamental period of observations is to be 12 months. Resolution of a 1-month cycle ($n = 12$), requires four samples per month, or sampling once per week.

APPLICATION OF THE FOURIER METHOD

In practice, the Fourier method described above must be adapted to each specific application. In addition to the minimum number of samples necessary in order to attain a desired resolution another restriction applies to variations in the sampling interval. Although the computer program used to obtain the results of this paper allows a varying sampling interval, thus accepting a sequence with missing observations, the sampling interval can be allowed to vary only within limits. For example, at least four samples per month are necessary to resolve a monthly cycle. This cycle will, however, not be resolved if the samples are taken on four consecutive days, rather than being evenly distributed throughout the month. It is also possible to aid the harmonic analysis in rapid convergence to its best fit with the observed values by adjusting the fundamental period of analysis and by performing some preliminary operations which are described below.

APPLICATION TO KOKO HEAD SEA-SURFACE TEMPERATURES AND SALINITIES

The sampling station is located near Koko Head at the exposed, eastern shore of Oahu so that the sea-surface temperatures and salinities measured there reflect open-ocean conditions. The salinities appear to be affected by runoff only on rare occasions of heavy rainfall. Both the temperatures and salinities are based on bucket samples. The salinity is determined in the Hawaii Area Fishery Research Center, Honolulu.

Before 1961 samples were collected at weekly intervals and subsequently twice weekly, usually on Tuesday and Friday mornings. Occasionally sampling has been missed. The computer program must therefore accept data with an irregular sampling interval.

The basic period for analysis has been chosen to be 1 year. Harmonic analysis began with the first sample and ended with the last sample of the year. The sampling time, in days and months, was converted to days of the year beginning with the first of the year.

Owing to a longer term trend, the value of a property at the beginning is not necessarily the same as at the end of an annual cycle. In the case of Koko Head salinities and Christmas Island temperatures, it will be seen later that an annual cycle is, in fact, not always apparent. The noncyclic trend during the analysis period can be obtained by linear approximation. Rapid convergence to the best fitting function can then be achieved by performing the harmonic analysis on the residuals of the observed values from a linear fit.

In our application the first observed value, $F(t_0)$, and the last observed value, $F(t_l)$, for the period were used to obtain the linear equation

$$S' = F(t_0) + bt$$

where $$b = \frac{F(t_l) - F(t_0)}{t_l - t_0} .$$

The residuals, $R_m = F(t_m) - [F(t_0) + bt_m]$, $m = 0,1,2, \ldots l$, were used to obtain the Fourier coefficients. The Koko Head temperatures and salinities for each year are then expressed by the function

$$S = K + bt + \sum_{n=1}^{k} C_n \cos\omega(nt - \alpha_n)$$

where $$K = F(t_0) + \frac{A_0}{2} \text{ and } \omega = \frac{2\pi}{l} .$$

The phase angles and coefficients for each of the years 1956-69 of the sea-surface temperatures are listed in appendix A Table 1, and of the sea-surface salinity are listed in appendix A Table 2.

The functions for each year together with the observed values of the sea-surface temperature and salinity have been drawn by automatic plotter and are presented in appendix B.

Quality control of the data was achieved by two passes of the data through the computer. First, the fitted graphs and plots of the observed values as well as listed deviations of observed values from the functions that resulted from the first computer analysis were used to reject obviously erroneous observations. The analysis was then repeated without the rejected observations. The tabulations in appendix A and figures in appendix B are the result of the second pass through the computer. The rejected values are plotted and identified in the figures of appendix B.

APPLICATION TO CHRISTMAS ISLAND SEA-SURFACE TEMPERATURES

The Christmas Island sea-surface temperatures are measured with a bucket thermometer each morning (about 0900 local time) in the channel leading from the open sea to the lagoon. The differences between open sea and lagoon water temperatures have not been determined. It is reasonable to assume that these temperatures differ, and so introduce variability in the observed temperature as tidal currents in the channel change from day to day. The tide-induced variability will, however, not be reflected by a harmonic function where the resolution of the highest harmonic is longer than 1 month. Although the sampling site is not ideal, the observed temperatures are believed to reflect, with some bias induced by lagoon temperatures, the changes of sea-water temperature from month to month.

The procedure to obtain functions of the Christmas Island temperatures was the same as that used for the Koko Head temperatures and salinities with the exception that a different fundamental period was chosen. In contrast to Koko Head where an annual cycle dominates the sea-surface temperature, longer term changes dominate the temperature at Christmas Island. The basic temperature pattern at Christmas Island also changes from year to year. For these reasons a duration of 120 days was chosen as fundamental period and Fourier analysis was performed, as before, on the residuals of the observed values from a linear fit.

For each year, the 120-day periods followed in sequence with an overlap of 30 days. The periods ran from the first day of the year to day 120, from day 91 to day 210, from day 181 to day 300, and from day 271 to day 390, extending 25 days into the following year. In this manner rapid convergence of the harmonic function to the best fit was obtained.

With daily sampling and a fundamental period of 120 days, harmonic analysis could be carried to the harmonic $n = 30$, but to do so would introduce variability that we wish to smooth out. Although a resolution of 1 month requires harmonic analysis to $n = 4$ only, the analysis was arbitrarily carried out to $n = 7$, resolving a period of 16 days.

The resulting phase angles and coefficients for 1954-69 of the sea-surface temperature are listed in appendix C. The functions for each year together with the observed values have been drawn by automatic plotter and are presented in appendix D.

Quality control procedures were identical to those for the Koko Head analyses. Relatively large data gaps occurred at Christmas Island in 1964, 1967, and 1968. Because some observations were available during each of the 120-day periods in question, harmonic analysis produced coefficients that enabled drawing of curves in appendix D although there were no data. These curves were not erased since it is instructive to see what harmonic analysis will do when faced with insufficient data.

DISCUSSION OF RESULTS

In this paper we are concerned with the derivation and presentation of harmonic functions of regularly observed sea-surface temperatures and salinities at fixed stations rather than with oceanographic interpretations. In the discussion of the results we will, therefore, concern ourselves primarily with the quality of fit of the functions. We will also briefly discuss some properties of the temperature and salinity distributions that are reflected by the functions and, finally, show functions spanning the entire time of observations.

QUALITY OF FIT

A superficial inspection of the figures in appendixes B and D shows that the harmonic functions follow the trend of the observed values very well. Closer inspection, however, reveals that there are cases where the fitted curves depart from the observed trend. An example occurred when the Koko Head salinity function (appendix B) for 1956 fluctuated about the observed values from day 145 to day 180. The fluctuations were caused by a data gap between these days. A 15-day data gap is too large when harmonic analysis resolves a period of 1 month. Another example of deviations occurred in the Christmas Island temperature function (appendix D) for 1968 between day 240 and day 275. Again, a 30-day data gap is too large when harmonic analysis resolves a period of 19 days.

These examples illustrate that the sampling interval in harmonic analysis may vary only within limits and that the interval of permissible sampling gaps depends upon the period resolved by the analysis. In cases such as were cited, where the fundamental period of analysis is much longer than the sampling gap, it is possible to constrain the harmonic function by inserting "dummy" values based on linear interpolation of the last sample before, and the first sample after, the data gap.

There are cases where the fitted curve fails to follow the observed trend. When the deviations from the fitted curves are relatively large, there is a tendency to reject the observed values during quality control procedures, blaming the deviations on erroneous sampling. Temperature deviations of this type occurred at Koko Head during days 65 to 90 of 1967. First the observed temperatures fell to 0.6° C below the fitted curve and then rose abruptly 1.3° to 0.6° C above the fitted curve. Erroneous sampling is ruled out since more than one sample was involved in establishing the trend that was abruptly broken and, in addition, the salinity showed similar variability during the same time interval. First the observed salinity rose to 0.15‰ above the expected value and then dropped abruptly 0.37‰ to 0.16‰ below the expected value. In the Hawaiian region the temperature increases and the salinity decreases southward. Thus, northward-southward displacements of the water that would result in the observed temperature and salinity changes were the probable cause for the large deviations rather than sampling error.

In order to assess the quality of fit quantitatively, we will consider several aspects of the standard error of estimate (root mean square deviations of the observed from the expected values). This statistical parameter is listed in three tables for each function, with harmonic analysis carried out for the fundamental period, the first harmonic, the second harmonic, etc. ($n = 1,2,3,\ldots$). Table 1 applies to Koko Head temperatures, Table 2 to Koko Head salinities, and Table 3 to Christmas Island temperatures.

In each case the listed standard error of estimate decreases or reaches a constant value with increasing n. The fit of the function therefore improves or levels off as the analysis is carried out to higher harmonics. Exceptions to this trend occurred in 1956, 1959, and 1961 when the standard errors of estimate for the Koko Head salinity functions (Table 2) increase as the highest n values are reached. Prior to May 1961, only four or five samples per month were obtained at Koko Head and therefore the highest n value permitted by the sampling frequency had been reached. In addition, sampling gaps occurred in 1956, as mentioned before, and in 1961 between days 220 and 241.

The fit of the Koko Head temperature functions (Table 1) improves most rapidly during the first few harmonics and with analysis carried out to $n = 6$, the standard error of estimate is near or below 0.3° C. With analysis carried out to $n = 13$, the standard error of estimate is below 0.2° C for all years excepting 1963 and 1965-68.

Greatest improvement of fit for the Koko Head salinity functions (Table 2) does not always occur during the first few harmonics but continues as analysis is carried beyond $n = 6$. In 1960, for example, the standard error of estimate with analysis to $n = 1$, $n = 6$, and $n = 13$, is 0.090‰, 0.075‰, and 0.038‰, respectively. The standard error of estimate at the n value of best fit in Table 2 is below 0.04‰ except

TABLE 1.—Standard error of estimate (° C) for each annual temperature function at Koko Head, 1956-68, with harmonic analysis carried out in sequence to $n = 1, 2, 3, \ldots$ and 13.

	N-VALUES												
YEAR	1	2	3	4	5	6	7	8	9	10	11	12	13
1956	0.21	0.19	0.17	0.16	0.16	0.14	0.14	0.14	0.13	0.13	0.12	0.12	0.11
1957	0.36	0.29	0.24	0.24	0.23	0.23	0.23	0.21	0.20	0.20	0.19	0.19	0.19
1958	0.31	0.29	0.24	0.24	0.22	0.22	0.22	0.22	0.22	0.20	0.20	0.18	0.17
1959	0.41	0.38	0.29	0.29	0.26	0.24	0.24	0.24	0.22	0.21	0.20	0.20	0.19
1960	0.33	0.27	0.24	0.24	0.23	0.23	0.22	0.22	0.19	0.19	0.17	0.16	0.15
1961	0.47	0.37	0.35	0.33	0.32	0.31	0.31	0.28	0.24	0.23	0.20	0.18	0.17
1962	0.32	0.25	0.25	0.25	0.23	0.21	0.21	0.21	0.20	0.19	0.19	0.19	0.17
1963	0.30	0.29	0.28	0.28	0.27	0.26	0.23	0.23	0.21	0.21	0.21	0.21	0.21
1964	0.29	0.29	0.28	0.25	0.25	0.24	0.23	0.21	0.20	0.18	0.17	0.17	0.16
1965	0.49	0.45	0.38	0.36	0.34	0.30	0.28	0.27	0.27	0.27	0.27	0.26	0.26
1966	0.43	0.32	0.32	0.30	0.30	0.27	0.27	0.27	0.26	0.26	0.26	0.26	0.26
1967	0.44	0.40	0.34	0.33	0.32	0.27	0.26	0.25	0.25	0.24	0.24	0.24	0.23
1968	0.37	0.32	0.23	0.28	0.28	0.27	0.26	0.25	0.24	0.24	0.23	0.23	0.23

TABLE 2.—Standard error of estimate (‰) for each annual salinity function at Koko Head, 1956-68, with harmonic analysis carried out in sequence to $n = 1, 2, 3, \ldots$ and 13.

	N-VALUES												
YEAR	1	2	3	4	5	6	7	8	9	10	11	12	13
1956	0.045	0.034	0.032	0.031	0.031	0.030	0.030	0.029	0.028	0.025	0.025	0.028	0.030
1957	0.068	0.057	0.054	0.054	0.045	0.041	0.039	0.037	0.034	0.034	0.032	0.030	0.030
1958	0.069	0.066	0.059	0.059	0.056	0.053	0.053	0.052	0.049	0.048	0.041	0.036	0.036
1959	0.174	0.099	0.076	0.075	0.074	0.073	0.069	0.064	0.060	0.058	0.054	0.053	0.054
1960	0.090	0.083	0.081	0.078	0.077	0.075	0.069	0.063	0.056	0.053	0.050	0.042	0.038
1961	0.064	0.061	0.051	0.047	0.043	0.038	0.037	0.036	0.033	0.030	0.027	0.028	0.028
1962	0.049	0.046	0.046	0.044	0.043	0.041	0.037	0.037	0.036	0.035	0.034	0.034	0.034
1963	0.054	0.053	0.052	0.046	0.045	0.045	0.043	0.041	0.037	0.037	0.034	0.033	0.033
1964	0.086	0.078	0.069	0.063	0.061	0.059	0.052	0.052	0.051	0.044	0.044	0.043	0.038
1965	0.094	0.085	0.078	0.072	0.072	0.071	0.066	0.064	0.058	0.056	0.054	0.048	0.045
1966	0.044	0.043	0.042	0.037	0.037	0.034	0.034	0.033	0.033	0.030	0.030	0.029	0.029
1967	0.079	0.078	0.074	0.072	0.068	0.061	0.055	0.054	0.052	0.051	0.050	0.046	0.044
1968	0.060	0.057	0.051	0.046	0.042	0.040	0.038	0.038	0.035	0.035	0.033	0.033	0.033

TABLE 3.—Standard error of estimate (° C) for each quarterly temperature function at Christmas Island, 1954-68, with harmonic analysis carried out in sequence to $n = 1, 2, 3, \ldots$ and 7.

		N-VALUES						
YEAR	QUARTER	1	2	3	4	5	6	7
1954	1	0.44	0.41	0.41	0.41	0.32	0.30	0.29
	2	0.36	0.35	0.33	0.33	0.32	0.30	0.30
	3	0.51	0.50	0.50	0.50	0.47	0.45	0.44
	4	0.39	0.38	0.37	0.33	0.30	0.30	0.29
1955	1	0.30	0.28	0.27	0.26	0.26	0.26	0.25
	2	0.29	0.29	0.28	0.28	0.28	0.27	0.26
	3	0.34	0.33	0.33	0.33	0.32	0.32	0.32
	4	0.46	0.45	0.43	0.42	0.40	0.39	0.39
1956	1	0.40	0.38	0.38	0.37	0.36	0.35	0.35
	2	0.52	0.50	0.48	0.48	0.46	0.45	0.45
	3	0.48	0.47	0.45	0.44	0.43	0.41	0.41
	4	0.38	0.38	0.36	0.36	0.36	0.32	0.32
1957	1	0.48	0.46	0.45	0.44	0.43	0.43	0.43
	2	0.61	0.54	0.54	0.54	0.53	0.51	0.51
	3	0.44	0.44	0.43	0.43	0.40	0.39	0.38
	4	0.40	0.39	0.35	0.34	0.33	0.30	0.28
1958	1	0.26	0.25	0.24	0.24	0.24	0.23	0.23
	2	0.33	0.33	0.33	0.32	0.31	0.30	0.29
	3	0.37	0.35	0.30	0.29	0.28	0.28	0.28
	4	0.32	0.31	0.28	0.27	0.26	0.25	0.25
1959	1	0.41	0.34	0.30	0.28	0.28	0.27	0.27
	2	0.40	0.38	0.36	0.35	0.34	0.34	0.33
	3	0.48	0.39	0.36	0.34	0.32	0.32	0.32
	4	0.43	0.39	0.36	0.32	0.31	0.29	0.29
1960	1	0.30	0.29	0.27	0.26	0.26	0.25	0.25
	2	0.35	0.33	0.32	0.31	0.31	0.31	0.31
	3	0.32	0.31	0.30	0.27	0.26	0.26	0.25
	4	0.39	0.32	0.29	0.26	0.26	0.24	0.23
1961	1	0.36	0.34	0.34	0.32	0.31	0.30	0.27
	2	0.34	0.34	0.31	0.27	0.26	0.26	0.25
	3	0.36	0.29	0.29	0.28	0.27	0.25	0.25
	4	0.26	0.24	0.22	0.22	0.20	0.19	0.18
1962	1	0.39	0.34	0.30	0.30	0.30	0.27	0.27
	2	0.38	0.33	0.31	0.30	0.29	0.27	0.25
	3	0.26	0.24	0.21	0.20	0.20	0.19	0.19
	4	0.30	0.26	0.25	0.24	0.24	0.24	0.24
1963	1	0.36	0.36	0.34	0.34	0.32	0.32	0.28
	2	0.46	0.38	0.31	0.30	0.28	0.27	0.21
	3	0.36	0.29	0.29	0.27	0.26	0.25	0.24
	4	0.30	0.29	0.28	0.26	0.26	0.25	0.25
1964	1	0.32	0.32	0.31	0.30	0.29	0.29	0.28
	2	0.37	0.31	0.30	0.29	0.28	0.28	0.27
	3	0.34	0.31	0.29	0.29	0.29	0.27	0.26
	4	0.28	0.27	0.26	0.23	0.22	0.21	0.21
1965	1	0.30	0.29	0.28	0.27	0.26	0.25	0.25
	2	0.36	0.32	0.29	0.29	0.29	0.29	0.29
	3	0.53	0.52	0.45	0.42	0.39	0.38	0.37
	4	0.37	0.32	0.31	0.30	0.30	0.30	0.29
1966	1	0.42	0.39	0.35	0.34	0.33	0.30	0.29
	2	0.42	0.35	0.35	0.34	0.34	0.31	0.30
	3	0.60	0.51	0.48	0.46	0.43	0.42	0.40
	4	0.68	0.68	0.53	0.52	0.51	0.51	0.47
1967	1	0.42	0.40	0.34	0.34	0.44	0.42	0.32
	2	0.40	0.39	0.36	0.36	0.36	0.36	0.35
	3	0.39	0.38	0.37	0.36	0.34	0.33	0.33
	4	0.32	0.30	0.28	0.27	0.27	0.27	0.26
1968	1	0.42	0.37	0.36	0.33	0.33	0.33	0.32
	2	0.37	0.35	0.31	0.31	0.31	0.30	0.30
	3	0.28	0.29	0.29	0.30	0.31	0.29	0.29
	4	0.28	0.27	0.26	0.24	0.23	0.21	0.21

in 1959 and 1965 when it is 0.054‰, and 0.045‰, respectively.

At Christmas Island (Table 3), the average standard error of estimate at $n = 4$ (resolution of 1 month) is near 0.33° C and therefore about 60 % higher than that for the Koko Head temperatures. As previously mentioned, high temperature variability is to be expected at the Christmas Island sampling site.

A standard error of estimate based on all samples used to obtain a function obscures the month-to-month changes in variability that may have occurred. At Koko Head the month-to-month changes in temperature variability as reflected by the standard error of estimate for each month ranges from 0.05° to 0.45° C, the same values for the Koko Head salinities range from 0.006‰ to 0.136‰, and those for Christmas Island temperatures range from 0.17° to 0.66° C. Assuming that sampling error remains constant, the range of variability reflects changes in oceanographic conditions.

The standard error of estimate computed from the temperature and salinity observations of each month also reflects sampling quality in that low values indicate the residual variability in the ocean plus sampling error. For the Koko Head temperature, low values of the monthly standard error of estimate are near 0.1° C and for the Koko Head salinity they are near 0.02‰. The sampling error is therefore within ±0.1° C for the temperature and ±0.02‰ for the salinity. These are the limits to be expected when bucket sampling of the temperature and salinity is carefully done.

Finally, how is the quality of fit affected by sampling frequency and how reliable are the expected values that may be obtained from the harmonic functions? The constraint imposed by the sampling frequency on the resolution that may be attained by harmonic analysis has already been discussed. The present question concerns improvement of fit when the sampling frequency is increased above the minimum requirements.

At Koko Head the sampling frequency was increased from once to twice weekly in 1961. No significant change can be seen in the standard errors of estimate listed in Tables 1 and 2 as a result of doubling the sampling frequency. This observation is consistent with results obtained from oceanographic data collected at Ocean Weather Station "P" in the Gulf of Alaska. Tabata (1964: Table 8) lists the monthly mean value and the standard deviation of the temperature at 10-m depth based on data obtained twice daily, data obtained every second, third, fourth, fifth, sixth, and seventh day of July 1959 and May 1961. For July 1959 the mean temperatures range from 10.70° to 10.81° C and the standard deviations range from 0.60° to 0.76° C. For May 1961 the mean temperatures range from 5.84° to 5.90° C and the standard deviations range from 0.39° to 0.46° C.

In May 1961 Koko Head temperatures and salinities were sampled on 25 days. The mean of all temperature observations was 24.67° C with standard deviation 0.27° C. The mean of temperatures taken every fifth day was 24.58° C with standard deviation 0.39° C. The mean of all salinity observations was 34.759‰ with standard deviation 0.051‰. The mean of salinities taken every fifth day was 34.772‰ with standard deviation 0.058‰. The temperature results from Koko Head are comparable to those from Ocean Weather Station "P" in that mean values and standard deviations based on different sampling frequencies fall within approximately the same range. The standard errors of estimate for the May 1961 Koko Head temperatures and salinities, based on the harmonic functions with resolution of 1 month, are lower than the standard deviations, namely, 0.25° C and 0.027‰, respectively. The standard errors of estimate as well as the standard deviations do not change significantly when the sampling frequency is increased above the required minimum to attain a desired resolution by harmonic analysis.

Increasing the sampling frequency does, however, improve the confidence limits of a mean value or the expected value of a harmonic function. A good measure of the confidence limits of a mean value is the standard error of the mean (the standard deviation divided by the square root of the number of samples). Return-

ing to Tabata's table the standard error of the mean for July 1959 is for twice daily sampling every day 0.086° C, and for twice daily sampling every seventh day 0.253° C. For the same sampling frequencies in May 1961 the standard errors of the mean are 0.053° and 0.15° C, respectively. For the May 1961 Koko Head temperatures the standard error of the mean is 0.055° C with 25 samples and 0.16° C with sampling every fifth day. The standard error of the mean for the May 1961 Koko Head salinities is 0.010‰ with 25 samples and 0.024‰ with sampling every fifth day. On the basis of these considerations, the expected values obtained from the temperature functions have an uncertainty of ±0.10° C, and those from the salinity functions have an uncertainty of ±0.015‰ when samples are obtained twice weekly.

At Christmas Island temperatures are sampled daily rather than twice weekly as at Koko Head. In consequence, despite the larger variability, expected values obtained from the harmonic functions have approximately the same uncertainty as those obtained from the Koko Head harmonic functions. This statement is confirmed by considering the error terms that can be obtained by taking the difference of the expected values at the midpoint of the 30-day overlap portion of the Christmas Island temperature functions (see appendix D). On average this error term is 0.07° C and ranges from 0 to 0.26° C.

SOME PROPERTIES OF THE TEMPERATURE AND SALINITY DISTRIBUTIONS

Although the harmonic functions are merely analytic expressions of the temperature and salinity as a function of time, they do provide, to some extent, insight into the nature of the distributions. For instance, the monthly standard error of estimate, mentioned in the previous section, provides a measure of the month-to-month changes in variability. At Koko Head there is no seasonal pattern in this variability of the temperature; however, there is a seasonal pattern in the variability of the salinity. The monthly standard errors of estimate of the salinity function with harmonic analysis carried out to $n = 13$, are listed in Table 4.

TABLE 4.—Standard error of estimate (‰) for each month, 1956-68, of the Koko Head salinity. Harmonic analysis is carried out to $n = 13$.

	MONTH											
YEAR	1	2	3	4	5	6	7	8	9	10	11	12
1956	0.030	0.017	0.027	0.048	0.064	0.052	0.024	0.008	0.012	0.014	0.014	0.015
1957	0.049	0.013	0.021	0.015	0.030	0.034	0.029	0.017	0.036	0.031	0.018	0.034
1958	0.006	0.041	0.052	0.049	0.059	0.026	0.028	0.028	0.013	0.023	0.044	0.022
1959	0.043	0.035	0.044	0.136	0.040	0.036	0.054	0.023	0.047	0.032	0.023	0.035
1960	0.042	0.032	0.018	0.019	0.056	0.043	0.075	0.035	0.033	0.014	0.014	0.024
1961	0.036	0.019	0.017	0.019	0.027	0.054	0.011	0.020	0.025	0.021	0.023	0.023
1962	0.054	0.040	0.064	0.021	0.013	0.023	0.025	0.033	0.031	0.031	0.018	0.027
1963	0.029	0.026	0.018	0.073	0.045	0.036	0.021	0.025	0.020	0.022	0.032	0.036
1964	0.031	0.033	0.031	0.030	0.029	0.019	0.035	0.050	0.053	0.052	0.024	0.036
1965	0.044	0.053	0.059	0.092	0.037	0.043	0.034	0.016	0.018	0.033	0.019	0.019
1966	0.026	0.016	0.011	0.014	0.022	0.021	0.011	0.012	0.016	0.033	0.065	0.036
1967	0.026	0.029	0.097	0.055	0.050	0.015	0.019	0.021	0.017	0.031	0.029	0.056
1968	0.034	0.024	0.057	0.041	0.040	0.019	0.035	0.026	0.018	0.021	0.016	0.038

In each year excepting 1957, 1964, and 1966, highest variability occurred during the first 7 months of the year. In 1957 a seasonal pattern was not clearly apparent and in 1964 and 1966 highest variability occurred during the last 5 months of the year. Although the seasonal pattern of variability has not been examined in detail, it is consistent with the results of previous studies (Seckel, 1962, 1969). First, Hawaii is located in the vicinity of a relatively high salinity gradient that delineates the boundary of the North Pacific Central Water. Thus, the salinity measured at the Koko Head sampling station is sensitive to variations in the location of this water type boundary. Secondly, northward displacement of water (warm advection) tends to occur during the first 7 months of the year. In consequence the water type boundary that generally lies south of the Koko Head sampling station during autumn and winter is brought to within the vicinity of the sampling station. The months with higher variability tend to be associated with declines in the Koko Head salinity.

Insight into the nature of the distributions is also obtained by examining the spectra of the harmonic functions. It is evident from the figures in appendix B, that considerable temperature and salinity variability at Koko Head occurs with timespans of 35 to 60 days. Rather than showing the amplitudes for each harmonic of every function, the 13-year mean of the absolute magnitude of amplitudes for each harmonic of the Koko Head temperature and salinity functions is presented in Figure 2.

For both the temperature and the salinity, the amplitude of the annual cycle ($n = 1$) is largest. The amplitudes then decline rapidly with increasing harmonics to $n = 5$. In the case of the temperature, a slight increase in amplitude occurs at $n = 6$ and $n = 9$. Similar small increases in amplitudes occur in the case of the salinity at $n = 7$ and $n = 9$. The increased amplitudes at $n = 6$ and $n = 7$, resolving 60- and 52-day periods, reflect the climatic signals described by Seckel (1962, 1969). The increased amplitude at $n = 9$, resolving a 41-day period, reflects shorter term variability that may be due to large geostrophic eddies with dimensions near 200 km (Wyrtki, 1967) or eddying flow near the Hawaiian Islands.

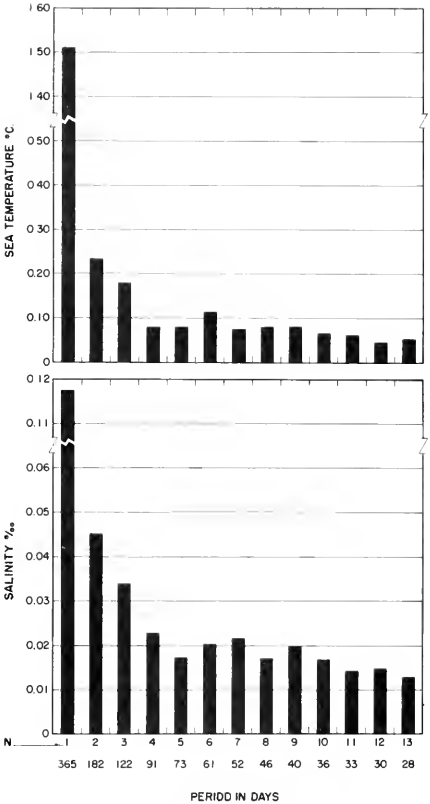

FIGURE 2.—Mean magnitude of amplitudes for each harmonic of the Koko Head temperature and salinity functions, 1956-69.

LONG-TERM HARMONIC FUNCTIONS

Long-term harmonic functions with the fundamental period spanning the entire duration of observations, can be obtained by the method described before in this paper. Temperatures and salinities were used as computed for the

1st and 16th of each month from the harmonic functions whose phase angles and coefficients are tabulated in appendixes A and C. Harmonic analysis was carried to $n = 42$ for the Koko Head temperature and salinity, and $n = 48$ for the Christmas Island temperature, giving in each case a 4 months' resolution. The fitted curves resulting from this analysis are shown in Figure 3, together with the values that were used as input data. Clearly the annual cycle forms the dominant signal in the Koko Head temperature curve. In the Koko Head salinity and Christmas Island temperature curves longer term changes are more pronounced than the annual cycle.

The relatively large deviations of the input data from the long-term function are to be expected. The figures of appendixes B and D show that variations with a duration of less than 4 months can be relatively large and are not resolved by the long-term analyses made.

The spectra of the long-term harmonic functions for the Koko Head temperatures and salinities and the Christmas Island temperatures are shown in Figure 4.

As is also apparent from Figure 3, the spectrum of the Koko Head temperature function is distinct from those of the Koko Head salinity and Christmas Island temperature functions. In the former the 12-month period has the most pronounced amplitude, but in the latter two, although the annual period has a large amplitude, the amplitudes of longer period changes are large and for some periods exceed those of the annual period.

CONCLUSION

The results of this paper show that sea-surface temperatures and salinities regularly monitored at island sampling stations can be expressed by harmonic functions of time. Advantages of analytic expressions for the temperature and salinity were cited in the introduction. Important applications will be in climatic oceanography where one may wish to filter out undesired "background noise." At Christmas Island, for example, the short-term variability with a duration of 1 month or less can be filtered out by using only the harmonic terms to $n = 3$ in the quarterly functions. At Koko Head, the variability with duration of less than 50 days, that may be due to large geostrophic or island-induced eddies, can be filtered out by using only the harmonic terms to $n = 7$ in the annual functions.

We mentioned in the introduction that the rates of change of temperature reflect the climatic processes of change and that distortions or aliasing may occur when monthly mean temperatures are used to compute the change of a property. Consider, for example, the Christmas Island temperatures from March to May 1968 (appendix D, days 61 to 152). In Table 5 are listed the monthly mean observed temperatures, the month-to-month changes of mean temperature, the expected temperatures from the harmonic functions for the 16th of each month (computed with harmonic terms up to $n = 4$), and the month-to-month changes of expected temperatures. It is clear from this illustration that the use of mean values would result in an underestimate of the rise in temperature from March to April, and would obscure the decline in temperature from April to May. The example is not isolated and other instances can be found in both the Koko Head and the Christmas Island data.

TABLE 5.—Month-to-month temperature differences using mean observed temperatures and expected temperatures from the harmonic function, Christmas Island, March to May 1968.

Date	Mean temperature	Change of mean temperature	Expected temperature	Change of expected temperature
	°C	°C	°C	°C
March 1968	25.1		25.1	
		0.9		1.2
April 1968	26.0		26.3	
		0.2		−0.3
May 1968	26.2		26.0	

The results also aid in the choice of an optimum sampling frequency. Both the desired confidence limit and the desired resolution must be considered. If the harmonic functions are to be used in monitoring the oceanographic climate as is the case of those presented in this paper, then the limits of about ±0.1° C for the expected temperature value and ±0.02‰ for the expected salinity value are adequate. As-

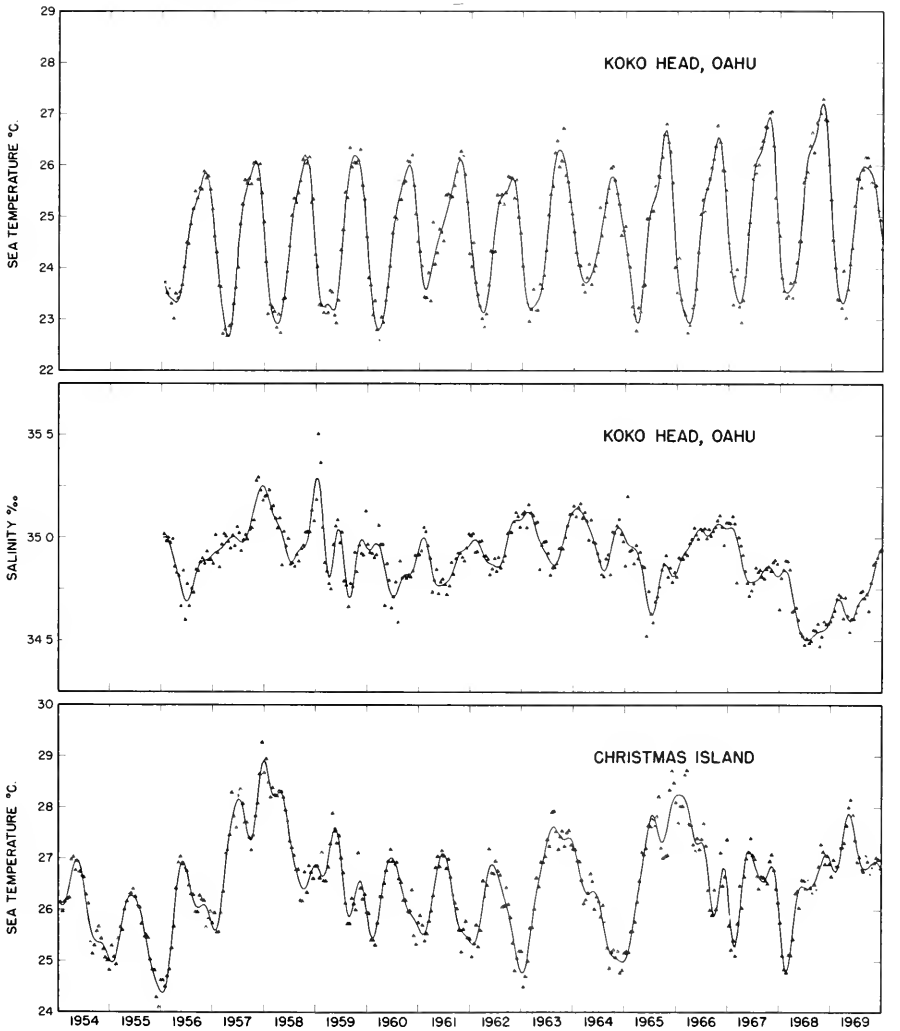

FIGURE 3.—Fitted curves with 4 months' resolution of the Koko Head temperature, 1956-69, Koko Head salinity, 1956-69, and the Christmas Island temperatures, 1954-69. Input data are indicated by small triangles.

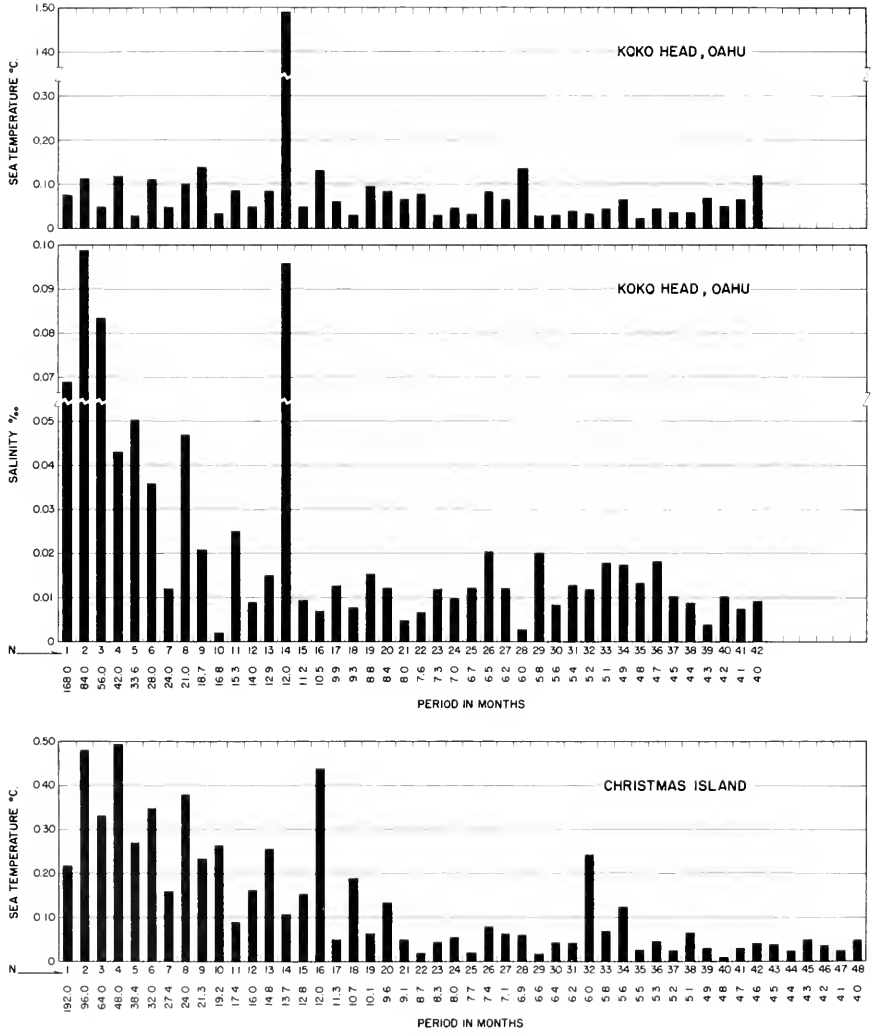

FIGURE 4.—Spectra of the long-term harmonic functions for Koko Head temperatures, 1956-69, Koko Head salinities, 1956-69, and Christmas Island temperatures, 1954-69.

suming that temperature and salinity samples are of Koko Head quality, then for a resolution of 1 month, weekly sampling is sufficient. Occasionally, however, a scheduled sample is not taken or an erroneous value must be eliminated. In such cases sampling gaps would become too large for the desired resolution. Undesirable sampling gaps can be avoided by doubling the minimum sampling frequency.

The simplicity and economy of deriving harmonic functions by computer are of practical value, particularly in the analysis of data sampled automatically. By this method large quantities of data can be brought into useful form rapidly.

The results of this paper, based on manual sampling, are useful in the investigations of changes with a duration of more than 1 month. Automated sampling would broaden the spectrum and permit analyses of shorter term variations such as diurnal changes, changes of tidal period, and other changes with durations of less than 1 month.

Automated sampling would also improve the quality of data since instruments can be placed in locations where undesirable variability is minimized and where manual sampling is difficult. At Koko Head, for example, samples are obtained from an exposed rock ledge where the island effects on the temperature and salinity are small. At Christmas Island, however, the sampling site is convenient and the best obtainable for manual sampling, but it is not the best in terms of monitoring open-ocean temperatures. This shortcoming is often also the case when temperatures and salinities are measured at tide stations located in protected bays or harbors.

The value of regularly monitoring the sea-surface temperatures and salinities has been demonstrated in many instances. For example, empirical relations between Koko Head temperatures and salinities and the availability of skipjack tuna to the Hawaiian fishery have been demonstrated (Seckel, 1963). Bjerknes (1969) has shown the relationship between anomalously high equatorial sea-surface temperatures using primarily Canton Island observations, and the intensification of the North Pacific westerlies and trades. This relationship must, in turn, affect temperatures and salinities in the North Pacific.

In view of these factors, serious consideration should be given to the establishment of automated sampling stations at selected islands in the Pacific. The derivation of harmonic functions, as demonstrated in this paper, would make reduction of data into usable form simple and economical and so facilitate the study of processes which govern the climate in both ocean and atmosphere.

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APPENDIX A

Sea-surface temperatures and salinities, Koko Head, Oahu, 1956-69: Phase angles and coefficients for harmonic functions

$$S = K + bt + \sum_{n=1}^{k} C_n \cos\omega(nt - \alpha_n) ,$$

$$\omega = \frac{2\pi}{365} \text{ days}^{-1} ,$$

t is the time in days beginning with the first day in each year.

APPENDIX A TABLE 1.—Phase angles and coefficients for sea-surface temperatures, Koko Head, 1956-69.

	PHASE ANGLES IN DAYS												
	N-VALUES												
YEAR	1	2	3	4	5	6	7	8	9	10	11	12	13
1956	73.88	69.97	10.42	-49.85	52.41	-25.23	70.98	-87.01	-55.97	19.33	-75.22	-52.34	46.35
1957	76.76	-72.88	30.75	58.99	8.81	-38.20	35.79	73.88	47.14	15.87	-36.68	-16.51	-8.30
1958	62.38	-6.51	-54.94	48.26	-34.27	-45.62	-51.72	-71.25	-46.58	-51.12	-10.61	-2.52	3.53
1959	65.59	-49.40	8.61	-1.48	56.56	-5.01	-70.18	17.77	71.89	-16.60	-27.85	-66.12	-50.78
1960	64.16	74.63	-62.69	37.38	-23.54	16.29	-89.39	23.77	-52.95	32.76	54.93	24.86	74.57
1961	53.19	15.31	-22.50	7.72	39.01	16.65	-51.44	-67.83	-2.26	49.83	88.27	-35.38	27.19
1962	66.15	-74.11	80.89	66.44	-7.78	-13.61	51.28	88.26	20.81	-30.04	29.78	41.65	-26.88
1963	63.76	-68.73	38.52	-27.45	-63.66	79.24	70.43	8.76	27.22	18.36	-76.61	35.19	-25.46
1964	73.31	-23.47	15.92	-57.15	-83.11	54.99	61.66	54.83	48.58	-0.50	51.76	3.89	-85.70
1965	64.85	45.82	-83.31	81.93	40.40	87.16	1.79	4.19	-21.73	-57.28	58.34	27.01	-90.88
1966	72.24	85.17	-29.44	82.13	-85.83	10.42	8.38	31.25	-0.28	-55.09	-24.99	65.54	-64.62
1967	60.48	66.72	-70.68	-9.91	83.89	84.03	-81.16	69.38	-9.63	-62.49	-84.89	-59.30	61.41
1968	65.18	71.07	-5.56	-19.37	26.00	-1.69	9.55	-41.85	-4.66	70.29	32.63	-19.50	-50.35
1969	58.78	-16.32	-68.76	90.78	49.97	12.98	89.93	40.17	-0.78	43.89	-88.96	-54.80	76.11
MEAN	66.13	62.61	-34.59	-9.48	68.75	40.82	29.88	86.20	-16.08	23.05	79.63	19.30	-87.99

APPENDIX A TABLE 1.—Phase angles and coefficients for sea-surface temperatures, Koko Head, 1956-69.—*Continued.*

			AMPLITUDES												
			N-VALUES												
YEAR	K	b	1	2	3	4	5	6	7	8	9	10	11	12	13
1956	24.2857	0.0014	-1.1174	-0.1340	-0.1099	-0.0633	0.0255	-0.1008	-0.0407	0.0206	0.0575	0.0346	-0.0565	0.0313	-0.0228
1957	25.1584	-0.0039	-2.0983	0.3004	-0.2188	0.0195	-0.1006	-0.0499	-0.0256	-0.1151	-0.0761	-0.0508	0.0691	0.0278	0.0400
1958	24.2433	0.0011	-1.4988	-0.1047	-0.2459	-0.0062	-0.0965	0.0225	-0.0385	-0.0154	0.0513	0.1033	-0.0374	-0.0878	-0.0477
1959	24.3697	0.0008	-1.5707	-0.2354	-0.3358	0.0470	0.1414	-0.1173	-0.0240	0.0239	0.0798	0.0968	-0.0969	0.0389	-0.0391
1960	24.3239	0.0008	-1.4963	-0.1570	-0.1641	0.0825	0.0383	-0.0437	0.0810	-0.0558	0.1175	0.0393	0.0931	-0.0677	0.1034
1961	24.7453	0.0006	-1.0587	-0.4112	-0.1832	0.1314	0.0498	0.1401	-0.0574	0.1761	0.1798	0.1026	0.1073	-0.1105	-0.0403
1962	24.7987	-0.0014	-1.4437	0.2802	0.0761	0.0502	-0.1250	0.1465	0.0561	-0.0798	0.0590	-0.0587	0.0302	0.0352	0.1037
1963	24.3471	0.0019	-1.4430	-0.1124	-0.1025	0.0501	0.0916	-0.0979	0.1870	0.0645	-0.1092	-0.0450	-0.0409	0.0344	0.0424
1964	24.7972	-0.0008	-1.0946	-0.0931	0.0691	0.1611	-0.0573	-0.0956	-0.0675	-0.1461	0.0639	-0.1162	0.0872	-0.0194	-0.0702
1965	24.8006	-0.0003	-1.6891	-0.2896	-0.3339	0.1715	0.1857	0.2120	0.1332	0.1040	0.0290	0.0596	0.0518	-0.0716	0.0622
1966	25.0047	-0.0014	-1.9152	-0.4056	-0.0944	-0.1330	0.0300	-0.1687	0.0236	0.0548	0.0825	-0.0161	0.0254	-0.0170	0.0253
1967	25.2850	-0.0008	-1.8725	-0.2850	-0.3005	-0.1125	-0.0966	-0.2437	0.1082	-0.0694	0.0794	-0.0341	0.0586	0.0555	-0.0572
1968	24.8546	0.0028	-1.5364	-0.2820	-0.2155	-0.0568	-0.0194	-0.0275	0.1192	-0.1263	0.0787	-0.0445	-0.0137	0.0294	-0.0817
1969	24.7701	0.0003	-1.3374	0.2035	-0.0760	-0.1235	0.1544	0.1096	0.1056	-0.0626	0.0594	0.1396	-0.1064	-0.0373	-0.0016
MEAN	24.6989	0.0001	-1.5040	-0.1458	-0.1291	0.0147	0.0220	-0.0164	0.0215	-0.0335	0.0461	0.0098	0.0324	-0.0135	-0.0013

APPENDIX A TABLE 2.—Phase angles and coefficients for sea-surface salinities, Koko Head, 1956-69.

	PHASE ANGLES IN DAYS												
	N-VALUES												
YEAR	1	2	3	4	5	6	7	8	9	10	11	12	13
1956	-14.52	-68.27	-89.13	-4.08	52.05	15.36	16.54	-2.09	-40.95	62.40	24.44	-7.01	-8.85
1957	-25.52	67.90	6.11	22.47	4.85	-25.00	-8.60	-19.40	28.27	-82.38	73.57	83.14	34.59
1958	25.99	46.89	57.99	50.30	15.25	-88.16	17.82	-10.99	20.65	-34.78	43.80	-31.33	-5.52
1959	-57.46	-68.08	18.94	-71.46	59.29	-35.64	-64.98	-19.12	-72.96	71.02	3.20	-8.24	28.66
1960	15.99	-61.03	81.05	56.42	-14.00	43.21	-58.49	-64.87	-87.20	62.22	71.57	4.93	-16.77
1961	-17.27	48.97	45.52	-26.18	2.69	13.58	2.15	-50.58	-38.94	82.68	-85.76	70.83	72.92
1962	-37.91	35.77	-1.00	-88.77	23.96	-32.29	-56.02	43.47	21.90	-52.99	24.88	-39.69	0.0
1963	17.33	-33.19	-66.85	53.84	13.09	-61.03	86.91	-72.22	12.17	2.06	-56.52	27.79	-39.95
1964	28.39	-19.79	-82.91	-82.99	53.70	-69.42	55.05	51.23	-17.95	8.29	-67.42	-80.98	-1.92
1965	-39.50	1.54	-49.50	-29.90	-31.57	-81.95	9.24	1.57	-56.55	90.65	42.78	-22.53	9.48
1966	-51.26	10.69	59.98	14.96	-10.51	78.69	26.93	-21.00	-86.73	30.96	10.93	-40.71	64.32
1967	-50.66	-58.57	35.70	38.98	-76.06	-77.32	-70.26	-26.09	17.82	-57.29	-13.46	26.17	44.48
1968	12.29	85.76	80.38	-11.84	-1.90	-31.29	-19.27	-49.39	-5.37	43.75	-66.43	70.00	18.69
1969	-9.16	30.08	30.65	-63.98	-47.50	47.99	74.49	66.13	-85.89	48.21	84.68	-58.03	-60.81
MEAN	-11.17	47.12	63.72	46.06	26.70	29.49	39.79	-16.95	-79.12	-76.28	-75.40	13.10	-5.70

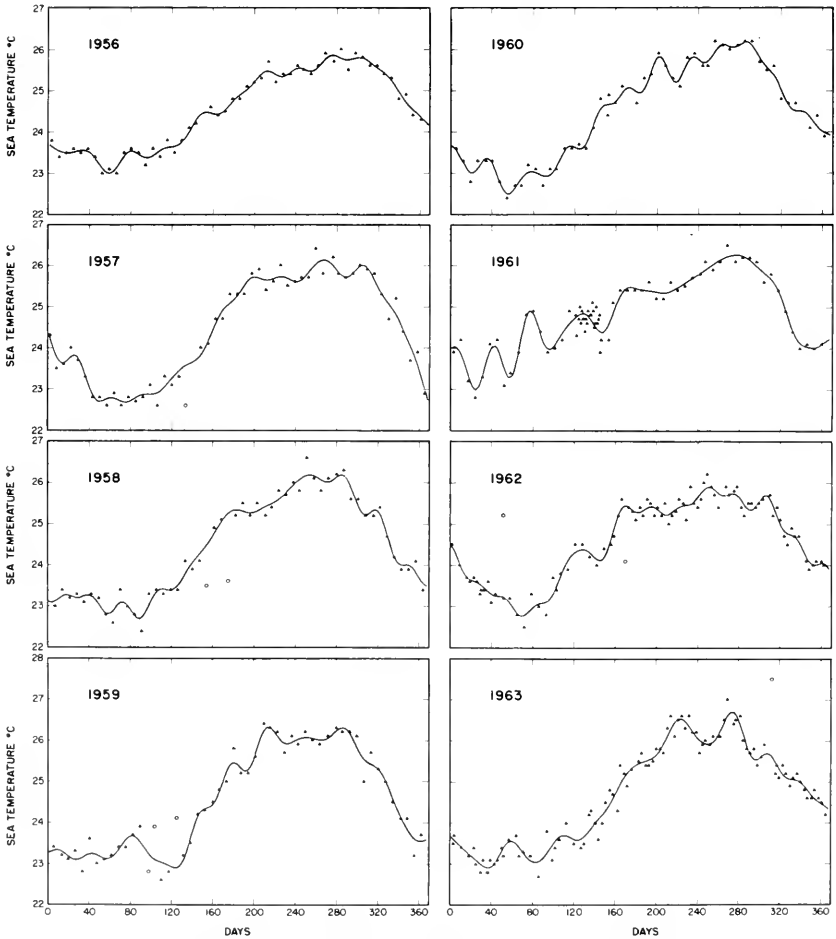
APPENDIX A TABLE 2.—Phase angles and coefficients for sea-surface salinities, Koko Head, 1956-69.—*Continued.*

			AMPLITUDES												
			N-VALUES												
YEAR	K	b	1	2	3	4	5	6	7	8	9	10	11	12	13
1956	34.8727	-0.0001	0.1261	-0.0420	-0.0164	-0.0071	0.0038	0.0099	0.0043	0.0167	-0.0176	-0.0258	0.0240	-0.0133	0.0270
1957	34.9345	0.0006	0.0604	-0.0544	-0.0305	-0.0029	0.0407	0.0242	0.0183	-0.0156	0.0195	-0.0079	-0.0127	0.0122	-0.0037
1958	34.9491	0.0004	0.1650	0.0324	0.0412	0.0088	0.0262	-0.0244	0.0063	0.0213	0.0244	-0.0130	-0.0292	0.0160	-0.0042
1959	35.2530	-0.0018	0.1996	0.2016	0.0884	0.0168	-0.0140	0.0120	-0.0272	-0.0340	-0.0271	0.0179	0.0254	-0.0191	0.0106
1960	34.8357	0.0000	0.1089	-0.0434	-0.0269	-0.0287	0.0239	0.0188	-0.0380	0.0378	-0.0373	-0.0198	-0.0187	0.0283	-0.0172
1961	34.8545	0.0001	0.1147	0.0368	0.0375	-0.0172	-0.0194	-0.0324	0.0162	-0.0191	-0.0179	-0.0177	0.0117	0.0064	-0.0151
1962	34.9396	0.0001	0.1084	-0.0230	-0.0116	0.0211	0.0035	0.0190	0.0254	0.0076	-0.0103	0.0106	-0.0051	0.0055	-0.0064
1963	34.9780	0.0001	0.1388	0.0067	-0.0164	-0.0371	0.0161	-0.0111	-0.0215	0.0162	0.0226	-0.0113	0.0197	0.0122	-0.0072
1964	34.9727	0.0001	0.1178	-0.0503	-0.0503	-0.0416	0.0209	-0.0158	0.0394	-0.0022	-0.0174	0.0316	-0.0025	-0.0142	0.0272
1965	34.9953	-0.0010	0.1566	-0.0564	0.0477	0.0412	0.0056	0.0163	0.0328	-0.0185	0.0356	0.0195	-0.0188	0.0331	0.0252
1966	34.8927	0.0006	-0.0401	-0.0131	0.0156	0.0271	0.0083	-0.0177	-0.0011	-0.0088	-0.0077	0.0161	0.0043	0.0084	-0.0114
1967	35.0184	-0.0009	0.1037	-0.0109	-0.0366	0.0248	-0.0310	-0.0417	-0.0368	-0.0184	-0.0169	0.0167	0.0091	0.0237	0.0179
1968	34.7135	-0.0006	0.1219	0.0424	0.0134	-0.0306	-0.0245	0.0191	0.0160	-0.0055	-0.0195	0.0044	0.0147	0.0050	-0.0022
1969	34.5912	0.0007	0.0821	0.0190	-0.0441	-0.0157	0.0044	-0.0243	-0.0200	0.0172	-0.0054	-0.0222	-0.0044	0.0103	0.0052
MEAN	34.9143	-0.0001	0.0972	-0.0051	0.0071	-0.0013	0.0064	0.0013	0.0070	-0.0026	-0.0065	0.0022	0.0073	0.0067	0.0040

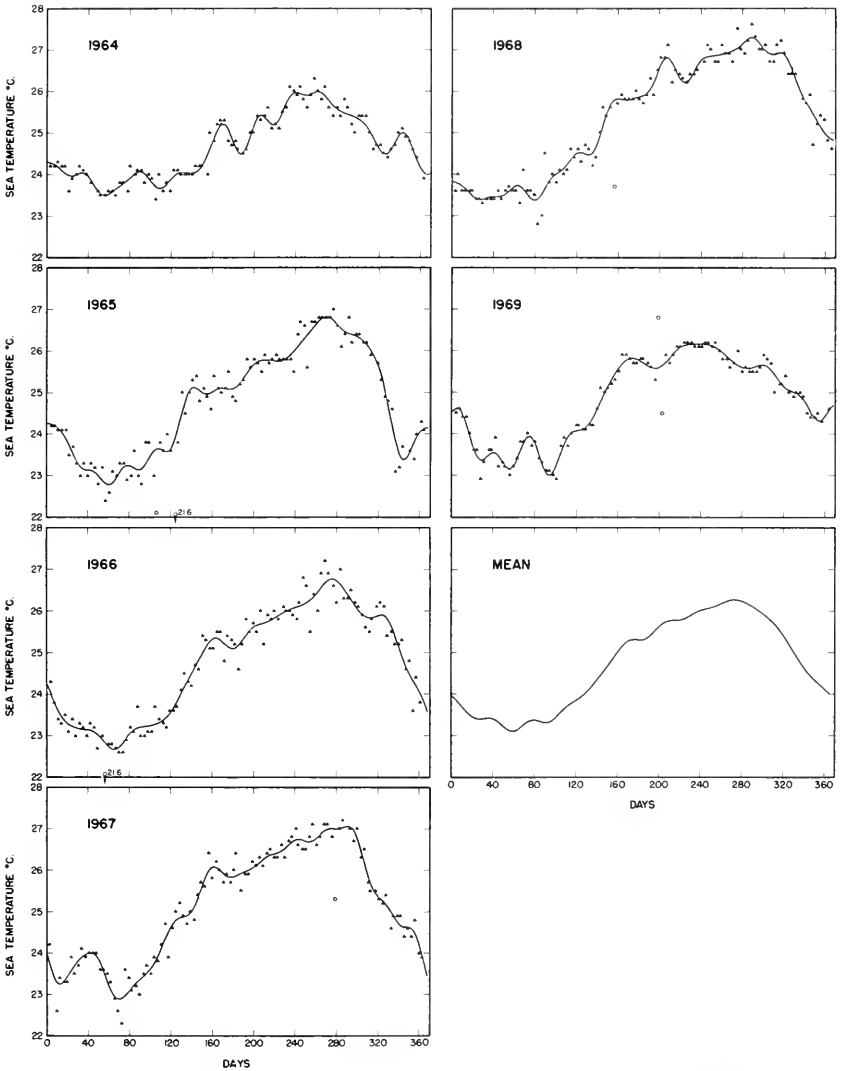
APPENDIX B

Sea-surface temperatures and salinities, Koko Head, Oahu, 1956-69: Fitted curves with observed values for each year.

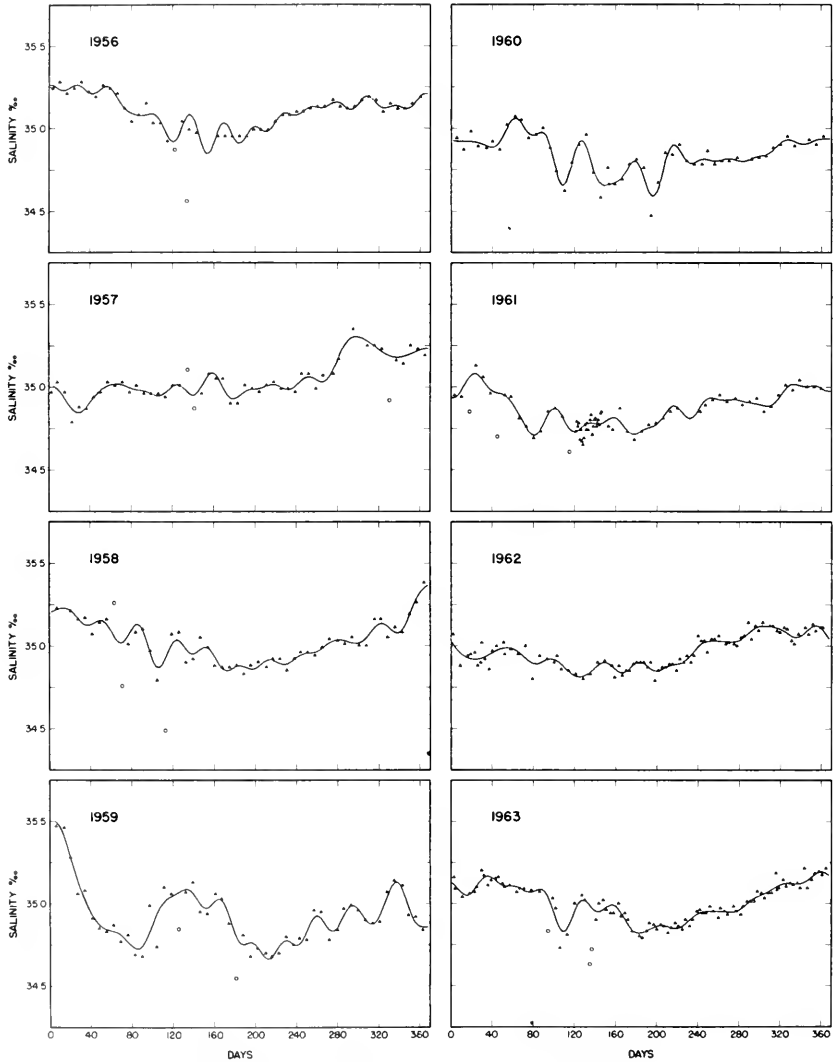
Note: Circled observations have not been used in the harmonic analysis.



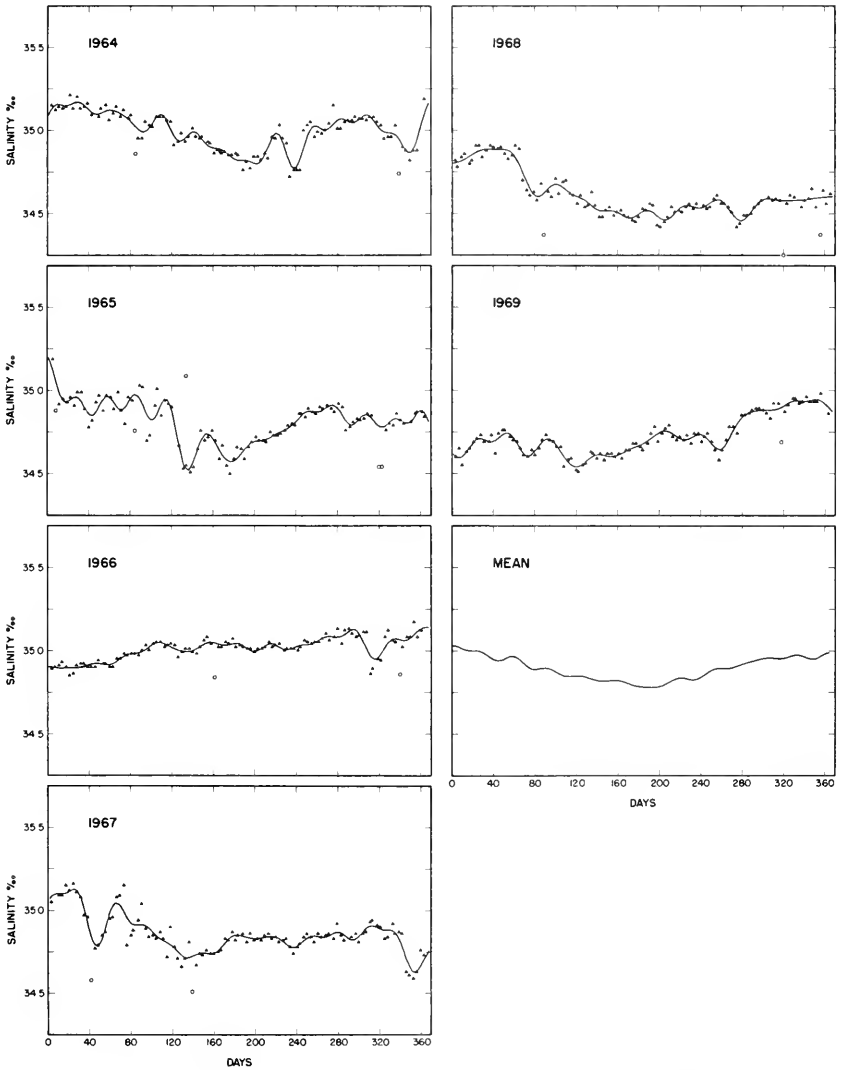
APPENDIX B FIGURE 1.—Sea-surface temperatures, Koko Head, 1956-69.



APPENDIX B FIGURE 1.—Sea-surface temperatures, Koko Head, 1956-69.—*Continued.*



APPENDIX B FIGURE 2.—Sea-surface salinities, Koko Head, 1956-69.



APPENDIX B FIGURE 2.—Sea-surface salinities, Koko Head, 1956-69—*Continued.*

APPENDIX C

Sea-surface temperatures, Christmas Island, 1954-69. Phase angles and coefficients for harmonic functions for each quarter of each year:

Days 1 to 120 = First quarter,
91 to 210 = Second quarter,
181 to 300 = Third quarter,
271 to 390 = Fourth quarter, extending 25 days into new year,

$$S = K + bt + \sum_{n=1}^{k} C_n \cos\omega(nt - \alpha_n) ,$$

$$\omega = \frac{2\pi}{120} \text{ days}^{-1} .$$

t is the time in days beginning with the first day of each quarter.

Note: Mean values do not include phase angles and coefficients for the third and fourth quarters of 1967.

APPENDIX C TABLE 1.—Phase angles and coefficients for sea-surface temperatures, Christmas Island, 1954-69.

PHASE ANGLES IN DAYS

		N-VALUES						
YEAR	QU.	1	2	3	4	5	6	7
1954	1	29.96	-14.98	0.68	22.60	-16.29	0.45	-28.43
	2	21.92	24.40	-25.77	15.21	23.02	24.75	8.06
	3	-19.46	21.45	-25.73	-10.27	-14.81	-6.52	-20.70
	4	-15.22	27.64	6.23	-9.84	25.21	27.55	-26.39
1955	1	-25.04	-14.36	-19.66	-25.05	10.26	1.57	-17.43
	2	-1.34	-22.96	16.15	-13.62	-27.83	-6.10	24.53
	3	-28.76	23.27	29.69	-21.77	-13.26	28.12	10.11
	4	-5.04	-11.73	-11.08	5.52	9.94	-2.89	-1.09
1956	1	-15.33	29.40	18.77	24.08	27.16	17.61	-20.53
	2	25.55	29.94	-23.88	27.13	-22.20	-10.40	-16.16
	3	-26.54	21.39	-22.54	13.46	20.79	29.39	-17.29
	4	-28.99	16.56	-15.87	-0.61	6.14	-27.68	1.48
1957	1	-20.33	9.65	-3.33	-13.34	-22.18	24.07	4.11
	2	12.45	3.80	-21.56	22.60	5.19	18.04	16.55
	3	12.50	-8.47	27.08	22.42	5.63	4.72	-24.37
	4	1.99	-11.91	-1.14	15.68	5.08	-17.77	-21.22
1958	1	-15.56	-9.55	16.21	-19.82	-16.02	-9.08	8.91
	2	-25.92	-8.31	-7.41	26.09	-26.16	-28.63	17.52
	3	17.11	-4.98	25.83	9.96	6.39	-15.05	-24.35
	4	-25.45	16.23	-11.73	19.38	7.25	16.83	20.96
1959	1	-11.25	18.21	25.30	27.40	2.42	-3.45	-27.76
	2	-4.58	-18.05	-2.17	-6.57	15.76	-29.98	28.66
	3	-13.92	-7.90	26.00	-0.14	15.14	3.09	21.42
	4	-25.63	-23.22	-13.08	18.13	-10.81	-22.26	24.72
1960	1	-21.36	3.16	-22.67	15.60	-1.93	22.75	21.91
	2	14.95	-26.09	18.68	6.43	23.12	-22.79	28.33
	3	21.14	17.18	10.04	7.87	6.28	20.84	-2.55
	4	-0.89	0.26	-28.94	9.46	17.99	23.48	-26.06
1961	1	-4.50	2.29	14.03	9.01	9.50	-26.75	14.56
	2	-9.38	-18.74	15.60	10.81	9.59	-18.31	-27.53
	3	-27.87	-27.65	27.26	26.80	21.47	-10.55	-13.26
	4	-19.95	-17.14	-3.05	0.39	-13.80	-7.92	24.95
1962	1	10.13	-24.96	-16.24	29.64	4.40	16.34	-1.98
	2	-10.33	-1.79	27.51	28.58	-0.81	3.38	-15.25
	3	18.78	20.95	-6.12	-29.12	-7.99	-1.52	2.56
	4	29.70	7.75	14.37	-13.15	-28.15	-4.47	13.65
1963	1	18.62	14.96	21.66	21.78	-21.72	-8.73	-19.16
	2	-25.86	-23.08	-21.84	-25.95	-12.90	-7.24	-3.15
	3	27.97	-11.92	17.92	-10.01	20.37	-9.25	15.06
	4	5.53	-27.74	-4.23	12.23	13.77	6.00	-14.49
1964	1	10.51	23.82	-4.95	-11.60	-22.14	13.52	9.47
	2	29.78	-24.66	11.64	-19.75	22.21	-13.85	-22.39
	3	3.66	-21.54	13.24	5.89	-17.78	16.09	6.84
	4	9.57	-19.61	-22.27	-11.16	-17.13	-18.46	-4.51
1965	1	-12.81	22.40	-29.54	24.56	25.12	16.28	-23.00
	2	-16.03	-23.97	-7.39	-19.45	2.93	-11.03	1.10
	3	28.09	-13.92	12.10	-9.47	6.00	14.42	-23.32
	4	-10.42	20.99	27.57	29.43	-14.90	17.87	-18.33
1966	1	-2.72	-22.51	-8.68	9.75	-10.62	-6.63	29.58
	2	29.77	26.12	4.26	21.28	15.28	27.61	-22.03
	3	7.34	25.04	9.12	25.58	1.93	10.76	4.92
	4	8.34	0.12	-20.67	1.30	-28.17	3.39	26.99
1967	1	-22.45	14.34	-25.09	18.95	0.56	13.45	-20.67
	2	-9.35	-8.88	-1.89	-22.17	-24.08	16.60	21.27
	3	-29.90	-2.24	18.31	26.99	-16.01	4.55	-12.24
	4	-19.32	23.12	7.45	-6.85	-19.61	7.19	8.69
1968	1	-9.14	-10.65	23.64	-20.93	11.66	-20.44	-24.41
	2	-14.96	-23.53	13.31	-14.18	7.83	27.96	20.00
	3	-0.07	-22.76	5.17	5.55	-23.92	-10.81	-2.39
	4	27.65	-15.84	3.23	-21.90	-10.98	-24.36	6.48
1969	1	13.34	14.46	6.54	-17.39	7.54	6.63	-7.29
	2	8.02	-23.81	-20.76	26.65	-28.91	29.11	27.75
	3	22.54	11.28	11.96	25.05	-8.17	12.49	29.02
	4	-24.65	-0.88	-15.33	-12.66	-11.06	-13.66	4.98
MEAN	1	-14.97	-10.80	-22.92	-20.97	16.82	20.22	20.72
	2	9.25	11.74	20.56	15.71	28.30	-19.45	-9.57
	3	5.57	22.63	15.62	14.65	25.53	22.30	-25.12
	4	-4.77	-2.64	-27.75	-26.43	20.79	-26.95	10.88

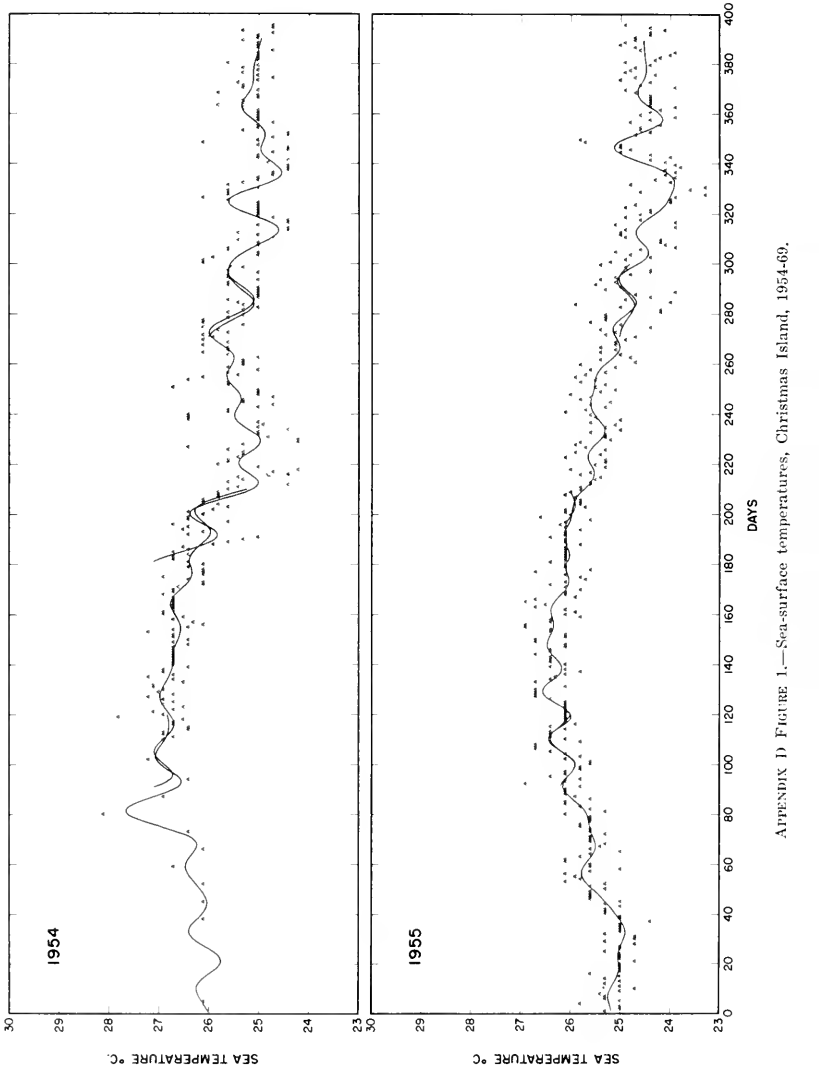
APPENDIX C TABLE 1.—Phase angles and coefficients for sea-surface temperatures, Christmas Island, 1954-69—*Continued.*

		AMPLITUDES								
				N-VALUES						
YEAR	QU.	K	b	1	2	3	4	5	6	7
1954	1	26.0740	0.0070	-0.2543	-0.1410	0.1609	-0.0772	-0.2429	0.0637	0.0743
	2	27.5957	-0.0169	-0.3583	-0.1443	0.1307	-0.1171	-0.1275	-0.1517	-0.0342
	3	26.4263	-0.0139	0.6131	0.0815	-0.0171	0.1382	0.2404	0.1908	-0.1111
	4	25.6919	-0.0090	0.2918	-0.1571	-0.0480	0.2517	0.1746	-0.1001	-0.0948
1955	1	25.1633	0.0067	0.2697	0.1614	-0.0873	0.1008	-0.0360	-0.0642	-0.0698
	2	26.2916	-0.0017	-0.1687	0.0292	-0.0565	-0.0347	0.0635	0.1000	0.1044
	3	26.1027	-0.0107	-0.0046	0.1350	-0.0375	-0.0219	0.0858	-0.0284	-0.0917
	4	24.8187	-0.0041	0.2259	-0.1449	0.1578	-0.1789	0.1436	0.0916	-0.0946
1956	1	24.3949	0.0183	0.4678	-0.1493	-0.0999	0.1311	-0.1107	-0.0674	0.0277
	2	27.7890	-0.0174	-0.4323	-0.2228	0.1648	-0.1209	0.1879	0.0861	0.0232
	3	27.0093	-0.0139	0.5433	-0.1689	0.1687	-0.1784	-0.1535	0.1329	0.0787
	4	26.1278	-0.0049	0.1022	0.0890	-0.1479	-0.0749	-0.0944	0.2481	-0.0168
1957	1	25.4577	0.0176	0.3612	0.1892	0.1458	0.1574	0.1045	0.0540	0.0693
	2	27.7558	0.0025	-0.2169	-0.4098	0.1621	-0.0802	-0.1359	0.2288	-0.1048
	3	27.6226	0.0008	0.4254	0.0970	0.0939	-0.1194	-0.2340	-0.1508	-0.1202
	4	28.0288	0.0066	-0.5153	0.0870	-0.2655	-0.1550	0.0453	0.2115	0.1496
1958	1	28.6567	-0.0050	0.1949	0.1052	0.0983	0.0407	0.0531	-0.0732	-0.0469
	2	28.2124	-0.0091	-0.2768	-0.0700	-0.0795	-0.0835	-0.1346	-0.0743	0.1074
	3	26.6129	0.0	0.3456	-0.1571	0.2536	0.0694	-0.1207	0.0260	0.0474
	4	26.2684	0.0066	-0.2862	0.0679	0.1355	0.1226	0.0867	-0.2355	0.0184
1959	1	26.8512	0.0025	0.3797	-0.3106	-0.2379	-0.1429	0.0562	-0.0545	0.0834
	2	27.8849	-0.0132	-0.2731	-0.1508	-0.1839	-0.1429	-0.0674	-0.0819	0.0641
	3	26.3895	-0.0025	0.5706	0.4088	-0.2084	-0.1502	-0.1315	-0.0472	-0.0216
	4	26.7237	-0.0091	-0.1536	-0.2531	-0.2372	-0.2151	0.1113	0.1418	-0.0299
1960	1	25.6504	0.0042	0.4722	0.0679	0.1461	0.1082	0.0721	-0.0691	0.0460
	2	26.9858	-0.0025	-0.3299	0.1787	-0.1260	-0.0797	0.0321	-0.0404	-0.0377
	3	26.5916	-0.0033	0.3107	0.1345	0.1430	-0.1676	-0.0941	0.0284	-0.0720
	4	26.0923	-0.0049	0.1670	-0.3030	0.1905	-0.1640	-0.0272	-0.1538	-0.1171
1961	1	25.3508	0.0109	0.3816	0.1665	0.0125	-0.1322	-0.1105	0.1068	0.1095
	2	26.7608	0.0025	-0.1900	-0.0611	-0.1699	-0.2098	0.1176	0.0254	0.0486
	3	26.5390	-0.0066	-0.4129	-0.2344	0.0796	0.0584	-0.1021	-0.1658	-0.0319
	4	25.9383	0.0016	-0.2387	0.1069	0.1592	-0.0197	0.1122	0.0496	0.0918
1962	1	24.9107	0.0168	0.1042	-0.2920	0.2226	0.0317	0.0329	0.1702	-0.0358
	2	26.5159	0.0033	-0.2921	-0.2701	-0.1616	0.1077	-0.0996	-0.1532	0.1131
	3	26.9093	-0.0139	-0.2714	0.1472	-0.1661	-0.0770	0.0533	0.0729	-0.0359
	4	25.6078	-0.0090	-0.2539	0.2136	0.0477	0.0963	-0.0275	-0.0509	-0.0641
1963	1	24.5106	0.0202	-0.1137	-0.0707	0.1670	0.0584	0.1332	-0.0370	-0.2257
	2	27.3700	-0.0033	0.5749	0.3627	0.3065	0.1371	0.1594	0.0779	0.2443
	3	27.2705	0.0041	0.3865	-0.2483	0.0802	-0.1328	0.1014	0.1157	-0.0641
	4	27.6762	-0.0066	-0.0697	0.1280	0.0433	0.1408	0.0486	0.0348	0.0603
1964	1	26.7596	-0.0025	0.4138	-0.0692	0.0988	0.1046	-0.0482	-0.0840	-0.0658
	2	26.2958	-0.0017	0.2579	-0.2425	-0.1394	0.0522	0.1147	0.0422	-0.0869
	3	26.0159	-0.0092	0.4285	-0.1814	0.1304	-0.0741	0.1106	0.0733	-0.1184
	4	24.8755	0.0033	0.1803	-0.1368	0.0999	0.1655	-0.0848	0.0989	-0.0399
1965	1	25.0849	0.0151	0.2248	-0.1215	0.0998	-0.0676	-0.1001	0.0939	-0.0430
	2	26.4776	0.0149	-0.2682	-0.2238	-0.1401	-0.0793	-0.0111	0.0295	-0.0471
	3	27.2809	0.0033	0.6393	0.1140	0.3702	0.2312	-0.2451	0.1376	-0.0732
	4	27.3759	0.0098	-0.7793	-0.2572	-0.0559	-0.1109	0.0833	-0.3606	-0.0654
1966	1	28.3584	-0.0076	-0.5145	0.1954	-0.2585	0.0741	-0.1413	0.1728	0.1227
	2	28.5148	-0.0198	-0.8788	-0.3261	0.0899	-0.0254	-0.0921	-0.1870	0.0975
	3	26.6145	-0.0041	0.4309	0.4345	0.2067	0.2310	0.2313	0.0932	0.1499
	4	27.2510	-0.0164	-0.8011	-0.0462	0.6174	0.1390	-0.1069	-0.0299	-0.2482
1967	1	25.3932	0.0076	0.3944	0.1627	0.2864	-0.0779	0.0240	-0.1438	0.0926
	2	26.4576	0.0066	-0.5433	-0.1618	-0.1932	-0.0712	-0.0620	0.0473	0.0553
	3	26.8436	-0.0044	0.1341	0.1093	0.0717	0.0649	-0.0496	-0.0822	0.0505
	4	27.8062	-0.0233	-0.0832	0.0851	0.0784	0.0593	0.0301	0.0046	0.0312
1968	1	25.1250	0.0033	0.5597	0.2691	-0.1413	0.2029	0.0708	0.0239	0.0730
	2	25.9567	0.0066	-0.1308	-0.1705	-0.2038	0.0706	0.0276	-0.1003	0.0172
	3	26.2971	0.0057	0.3013	0.0743	-0.1219	0.2030	0.1886	-0.2381	0.2042
	4	26.9967	0.0	0.1068	-0.0967	-0.1344	0.1439	-0.1141	-0.1165	0.0213
1969	1	26.4649	0.0168	0.1895	0.1321	-0.0869	-0.1854	0.0663	-0.0638	0.0926
	2	28.5357	-0.0165	-0.2815	0.3460	0.1314	-0.1603	0.1393	-0.1347	-0.1080
	3	27.0945	-0.0025	-0.0625	0.1304	-0.0853	-0.1166	-0.0388	0.1949	0.0671
	4	26.4789	0.0074	-0.2169	-0.0886	-0.1190	-0.1058	-0.1537	-0.0858	-0.0390
MEAN	1	25.8878	0.0092	0.2151	0.0484	0.0522	0.0285	-0.0074	-0.0107	0.0132
	2	27.2124	-0.0041	-0.1744	-0.0779	-0.0930	-0.0509	-0.0233	0.0283	0.0229
	3	26.7124	-0.0044	0.1770	0.0789	0.0498	-0.0071	-0.0629	0.0384	-0.0094
	4	26.3568	-0.0019	-0.0997	-0.0317	0.0509	0.0459	0.0341	0.0573	-0.0145

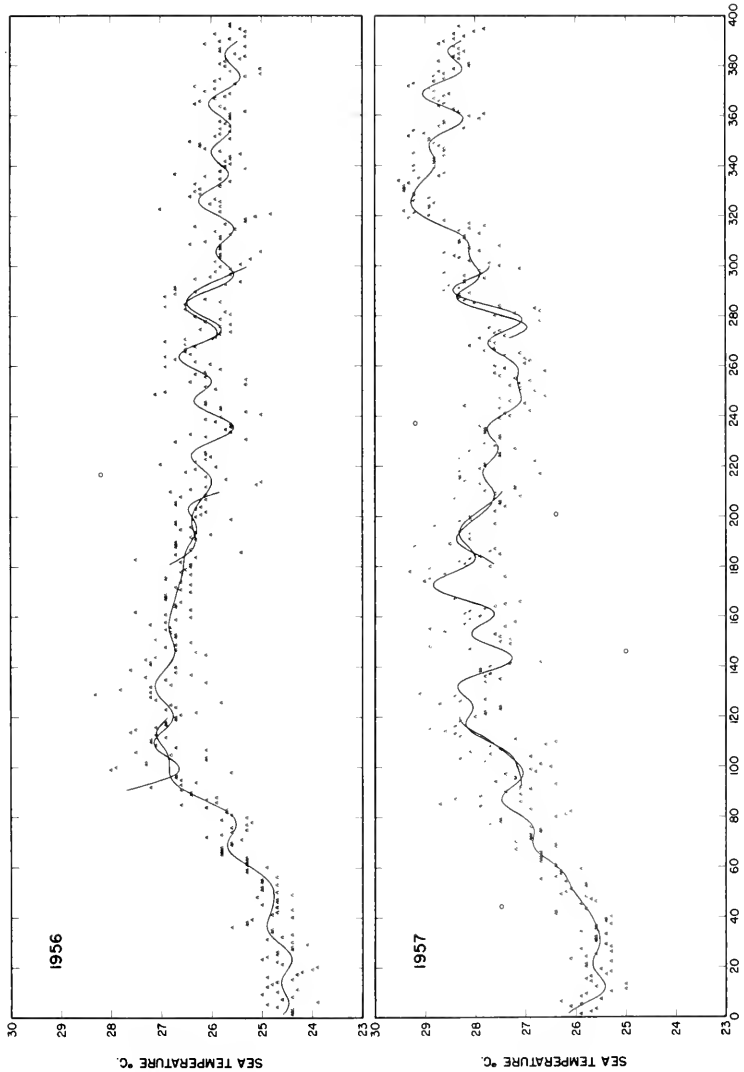
APPENDIX D

Sea-surface temperatures, Christmas Island, 1954-69: Fitted curves with observed values for each year.

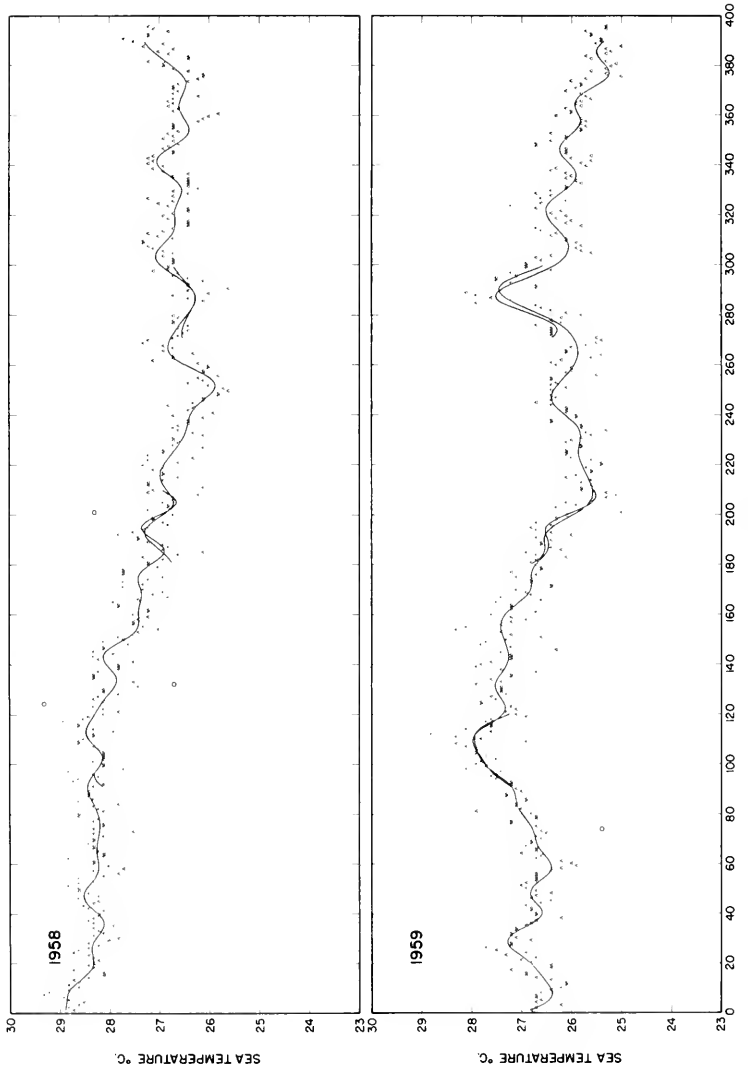
Note: Circled observations have not been used in the harmonic analysis.



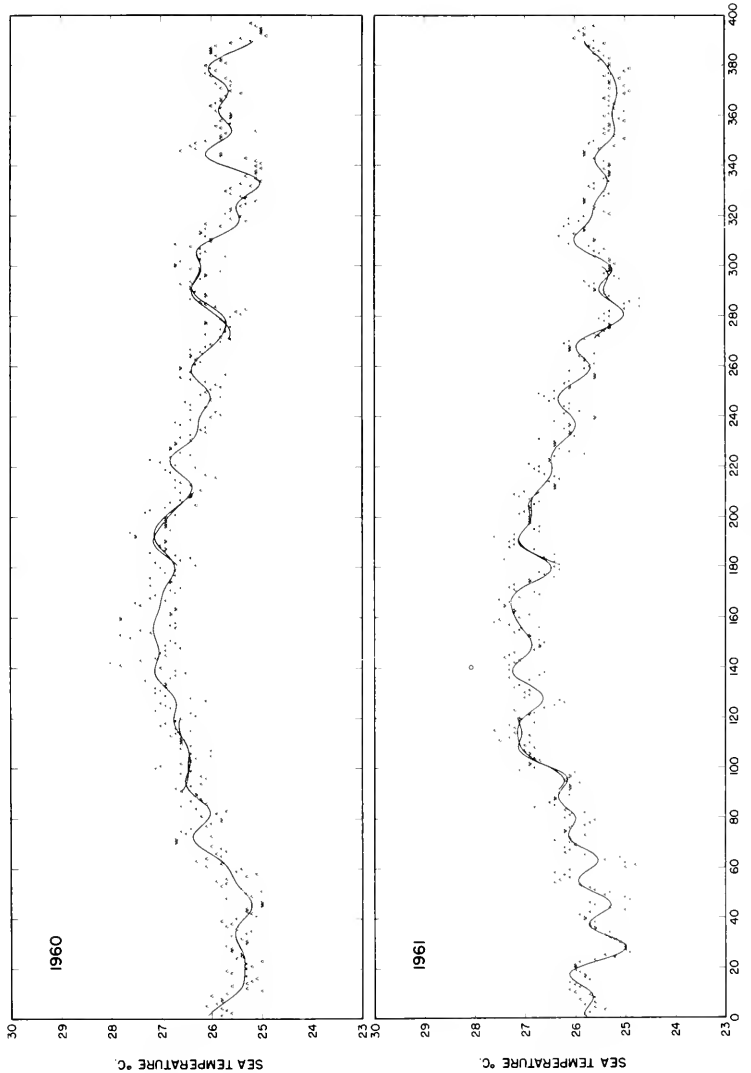
APPENDIX D FIGURE 1.—Sea-surface temperatures, Christmas Island, 1954-69.



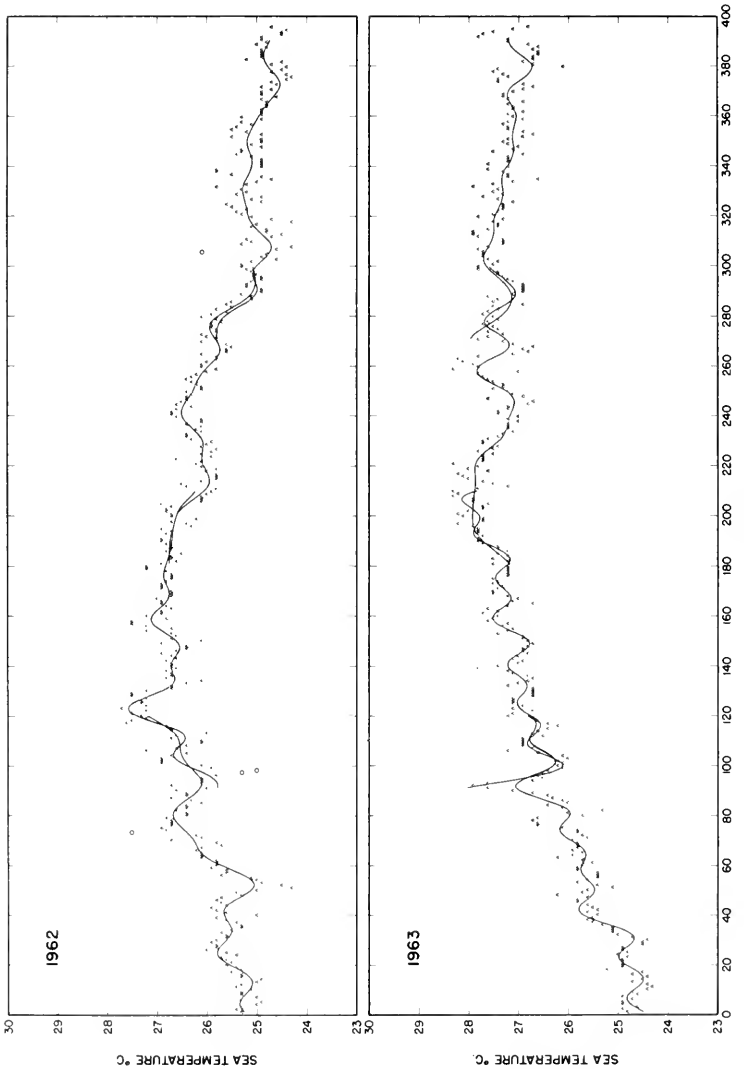
APPENDIX D FIGURE 1.—Sea-surface temperatures, Christmas Island, 1954-69.—*Continued.*



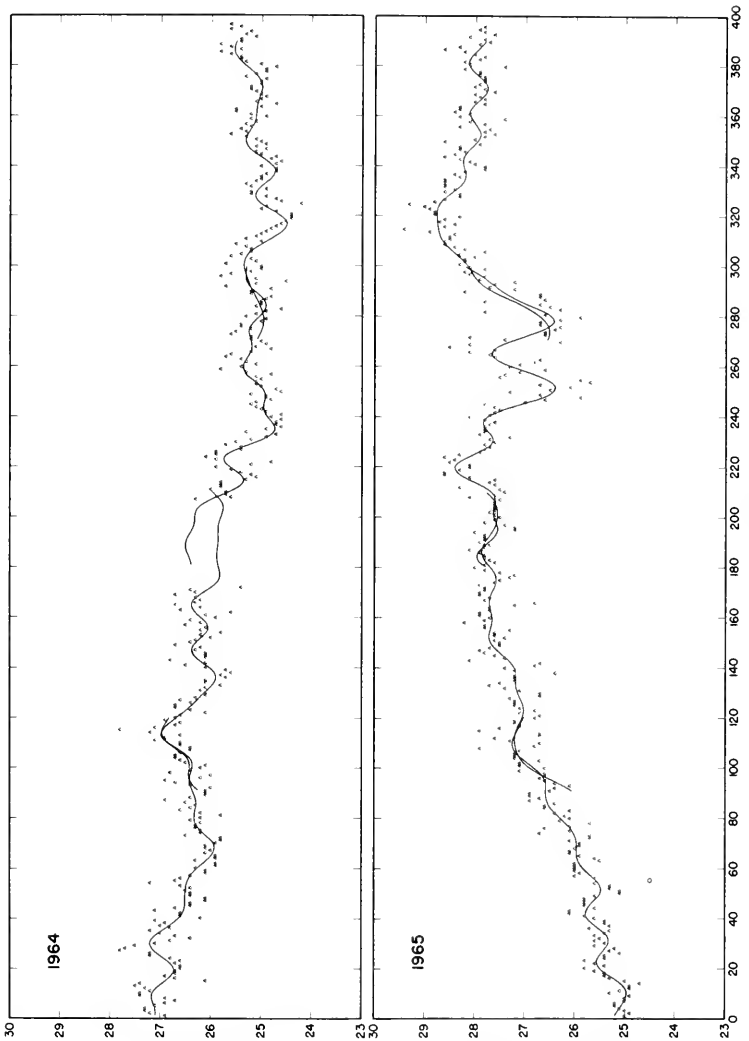
APPENDIX D FIGURE 1.—Sea-surface temperatures, Christmas Island, 1954-69.—*Continued.*



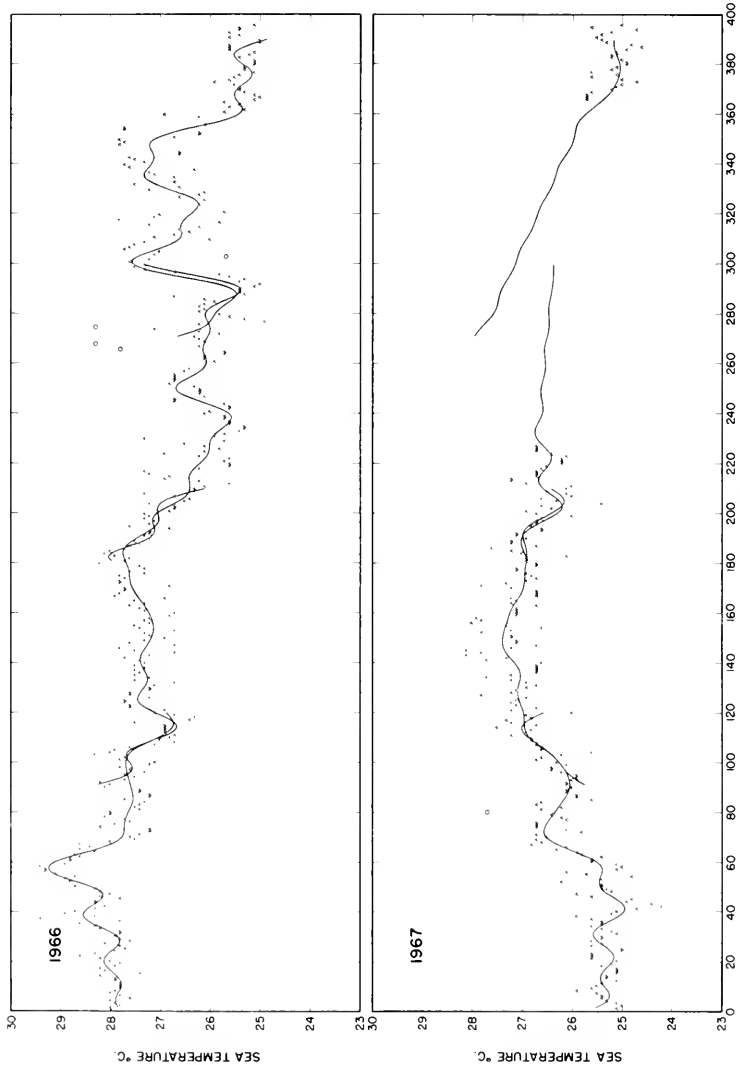
APPENDIX D FIGURE 1.—Sea-surface temperatures, Christmas Island, 1954-69.—*Continued.*



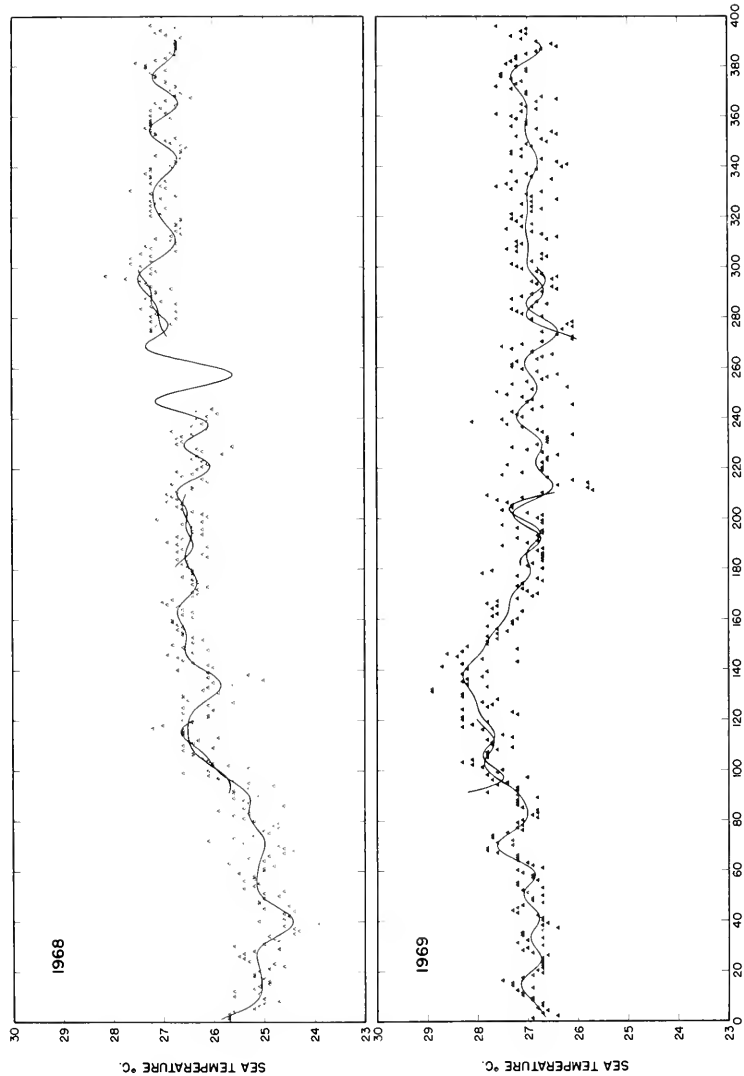
APPENDIX D FIGURE 1.—Sea-surface temperatures, Christmas Island, 1954-69.—*Continued.*



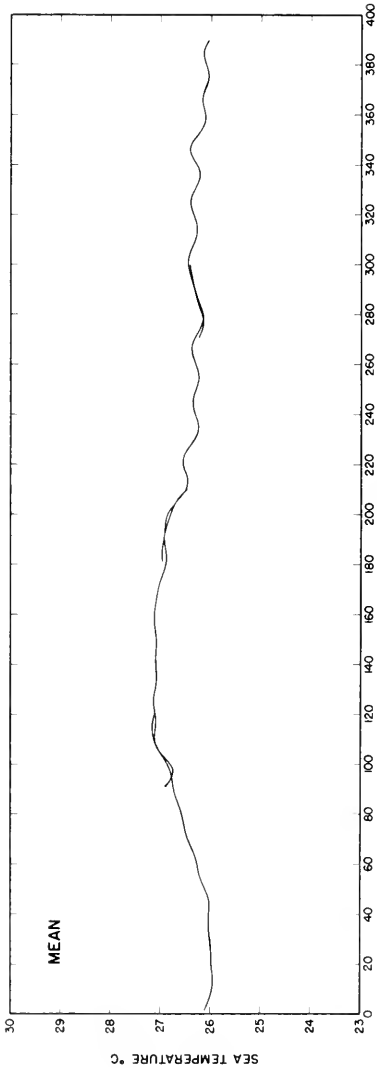
APPENDIX D FIGURE 1.—Sea-surface temperatures, Christmas Island, 1954-69.—*Continued.*



APPENDIX 1) FIGURE 1.—Sea-surface temperatures, Christmas Island, 1954-69.—*Continued.*



APPENDIX D FIGURE 1.—Sea-surface temperatures, Christmas Island, 1954-69.—*Continued.*



APPENDIX D FIGURE 1.—Sea-surface temperatures, Christmas Island, 1954-69.—*Continued.*

MASKING UNDESIRABLE FLAVORS IN FISH OILS[1]

GISELA JELLINEK[2] AND MAURICE E. STANSBY[3]

ABSTRACT

The odor of fish oil used medicinally may require masking by flavoring materials. During a search for suitable masking materials, fresh, specially refined menhaden oil having a minimum of flavor was stored with and without added flavoring materials for 5 days at 75° F and for longer periods at several lower temperatures. In initial preliminary screening tests with 66 different flavoring materials, the masking of rancid or other unpalatable flavors developing in the stored oil was evaluated by a small panel. In later tests, a large consumer-type panel consisting of untrained laboratory personnel was used to determine the preference for the flavors of those materials that worked best in the screening test and that were of a type approved by the U.S. Food and Drug Administration for use in foods. Several flavoring materials showed promise, particularly those having the flavor of root beer, lemon, wintergreen (methyl salicylate), and wild cherry.

When fish oil is used medicinally—for example, as a cholesterol depressant or as a source of vitamins—the presence of "fishy," rancid, or other flavor components may make it unpalatable. One way of overcoming this problem is to add some flavoring material that will mask the undesirable flavor. It is important that the added flavor be one that is pleasing to most consumers so that we do not merely substitute one undesirable flavor for a slightly more undesirable one. The aim of this work therefore was to find flavoring materials, approved for food use, that would adequately mask the unpleasant flavor components developing in stored menhaden oil (the fish oil produced in largest quantity in the United States) and that would also have a flavor pleasing to most customers.

We carried out this study in two experiments. In Experiment 1, reasonably palatable menhaden oil refined by a special technique was stored for 5 days at room temperature with and without added flavoring materials. A small trained panel was used to determine the efficiency of over 66 different flavoring materials in masking the developing undesirable flavor components in the stored oil. Then, in Experiment 2, those materials giving the best masking action, excluding any not approved for use with food, were further tested by a larger consumer-type panel for hedonic rating of the different flavors resulting from adding the selected flavoring materials to the oil before it was stored.

EXPERIMENT 1—MASKING TESTS

The first series of tests were set up as a rapid screening of many flavoring materials to see which ones best masked the undesirable off flavors that develop in stored menhaden oil. At this stage, no consideration was given to individual preference for the flavor additive, which was investigated in Experiment 2.

MATERIALS

Menhaden oil was specially refined by a combination of clay bleaching, molecular distillation, and treatment with massive quantities of silica gel (Stansby and Jellinek, 1965). This treatment yielded an oil that was free of fishy and rancid flavor components but that still retained some small burnt flavor.

Sixty-six flavor additives that were screened initially included synthetics and isolates,

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undiluted and in alcoholic solution, essential oils, and imitation flavoring compositions. The additives were obtained from many sources.

METHODS

In the initial work, 5-ml samples of menhaden oil, with or without flavor additive, were held in covered plastic petri dishes (100 by 15 mm) for 5 days at 75° F (22° C). Preliminary tests indicated that 1 part by volume of flavoring material per 400 parts by volume of oil gave about the proper concentration with most flavor preparations, so this concentration was used throughout the work. With the large surface of oil exposed to air in this test, a fairly advanced stage of rancidity was reached in 5 days.

In some of the later work in which larger samples were required, oil in both 200-ml (8-oz) and 25-ml (1-oz) stoppered bottles was stored: (1) at 40° F (4° C) for 8 to 12 weeks, (2) at —20° F (—29° C) for 8 to 12 weeks, and (3) at room temperature—that is, at 75° F (22° C) for 4 weeks.

For various aspects of this work, especially in preliminary observations, paired comparison tests, duo-trio tests, ranking tests and descriptive tests—all described by Jellinek (1964) were used. For most of this work, however, descriptive tests and ranking tests were used; only the descriptive and ranking tests will be tabulated in this report.

Samples of oil were tasted by immersing the tip end of a plastic spoon into the oil and placing the spoon into the mouth. This procedure avoided coating the lips with oil, and it eliminated much of the objection of some panel members to an undesirable oily feel during protracted examination of many samples.

The panel used in this part of the work consisted of 5 to 7 laboratory workers all of whom had had some previous experience with odors and flavors of fish oils. Several preliminary training sessions were held in order for them to become familiar with and be able to use consistent terminology for the odors and flavors to be encountered in the experimental work.

RESULTS

Initial Screening Test

In an initial screening test of all 66 flavoring compounds, a degree-of-masking scale was used as follows: ++, very good masking; +, good masking; ±, good masking when first tasted but did not mask aftertaste; ?, questionable masking; —, negative masking—that is, the taste was not masked (Table 1). The flavoring materials are listed in alphabetical order within each category.

Main-Examination Test

We then made a more detailed examination, paying greater attention to the type of flavor which sometimes varied at different stages during the storage of the oil. The following comments show certain limitations of many of the flavor additives.

Flavoring materials with "green" or "floral" flavor properties.—Because fresh fish oil has a "green-grassy" or "green-cucumber" odor and flavor, two green standards had been worked out in earlier experiments (Stansby and Jellinek, 1965). These standards were cis-hexen-3-ol-1 and Green Aroma Hr (coded sample of Haarmann and Reimer, Holzminden, West Germany,[1] probably: nona-2-enal or nona-2-dienal or a mixture of both (Forss, Dunstone, Ramshaw, and Stark, 1962). These two green standards were tried as masking agents.

Cis-hexen-3-ol-1 contributed a definite green-grassy note to the menhaden oil and also showed very good masking abilities in the exposed menhaden oil.

Green Aroma HR contributed a green-cucumber note to the fish oil but seemed to have prooxidant qualities. In comparison with the control sample, the menhaden oil got rancid faster and reached a higher intensity. These observations seem to be similar to those made about 20 years ago on butter with a high content of di-

[1] The use of trade names is merely to facilitate descriptions; no endorsement is implied.

TABLE 1.—Effectiveness of 66 flavoring materials in masking the fishy flavor and odor of menhaden oil.

Category and substance	Flavor type and descriptions	Masking effect
Synthetics and isolates		
Acetoin	butterlike	±
Agrumen aldehyde	fruity-citrus	−
Anethol, U.S. Pharmacopoeia (U.S.P.)	spicy-anise	++
Benzaldehyde	sweet-bitter almond	−
i-Butyl quinoline	green-spinachlike or earthy (asparagus)	−
Carvol	spicy-caraway	−
Cinnamic aldehyde	spicy-cinnamon	?
Citral	fruity-lemon	±
Ethyl phenyl acetate	sweet-honeylike	+
Ethyl salicylate	wintergreen	++
Eucalyptol	cooling	−
Eugenol	spicy-cloves	−
Foretine	green-mushroomy/earthy	−
Geraniol	green-leafy & floral-rosy	−
Geranyl acetate	green-leafy & floral-rosy	−
Geranyl butyrate	green-leafy & floral-rosy	−
Geranyl propionate	green-leafy & floral-rosy	−
cis-hexen-3-ol-1	green-grassy	++
i-Jasmone	green-leafy	−
Linalool	floral	−
Melonol (2,6-dimethyl-5-hepten-1-al)	fruity-melony	+
Menthol	cooling	−
Methyl nonyl ketone	green-?	−
Methyl salicylate	wintergreen	++
Styralyl acetate	green	−
-Undecalacton	fruity-peach	−
Vanillin	sweet-vanilla	?
Essential oils		
Anise oil, U.S.P.	spicy-anise	++
Bay oil	spicy-bay	−
Caraway oil	spicy-caraway	−
Cassia oil	spicy-cassia	++
Cinnamon oil, U.S.P.	spicy-cinnamon	++
Clove oil	spicy-clove	−
Ginger oil	spicy-ginger	−
Essential oils-Con.		
Lemon oil, U.S.P.	fruity-lemon	+
Lime oil	fruity-lime	+
Orange oil, U.S.P.	fruity-orange	+
Peppermint oil	cooling-peppermint	−
Root beer oil	wintergreen group	++
Spearmint oil, rectified	cooling-spearmint	−
Imitation flavoring compositions		
Blood Orange Flavor	fruity-orange	+
Imitation Boysenberry Flavor	fruity-boysenberry	−
Butter Aroma	butterlike	±
Cherry Bouquet	fruity-cherry or sweet cherry	++
Imitation Cranberry Flavor	fruity-cranberry	−
Fruity Bouquet	fruity	++
Green Aroma HR	green-cucumber	+
Imitation Honey Flavor	sweet-honeylike	+
Imitation Lemon Juice Flavor #51.124A	fruity-lemon	+
Lemon Mint Flavor	fruity-lemon	+
Lime Mint Flavor	fruity-lime	+
Imitation Melon Base	fruity-melon	±
Orange Mint Flavor	fruity-orange	+
Tetrarome Orange	fruity-citrus	+
Violet Flavor	floral-violet	−
Imitation Essence Arrack	fruity-brandy	−
Imitation Butterscotch	sweet-caramel	?
Oil Brandy Flavor	brandy-like	−
Imitation Essence Caramel	sweet-caramel-vanilla	?
Imitation Oil Soluble Flavor Wild Cherry #12009	fruity-cherry	++
Imitation Oil Chocolate	sweet-caramel-chocolate	?
Imitation Essence Cocoa	sweet-malt-chocolate	?
Malt True Concentrate	sweet-malt	−
Imitation Aroma Maple	sweet-maple	−
Imitation Oil Rum, Jamaica #017	fruity-rumlike	++
Tutti Frutti Oil	fruity-candy	++

acetyl. Butter with a high diacetyl content has more butter flavor and turns rancid faster than does butter with a low diacetyl content. For this reason, export butter has to be washed carefully to lower the content of diacetyl (Moncrieff, 1951).

In addition to these two green standards, some other chemicals with green character or floral character, or both, were tried but without success. They are listed among the synthetics and isolates above. Some of them—namely, i-butyl quinoline, foretine, i-jasmone, methyl nonyl ketone, and styralyl acetate—contributed sharpness and a biting note to the menhaden oil.

Geraniol, geranyl acetate, geranyl butyrate, and geranyl propionate contributed a green, somewhat leafy note combined with sharpness. In addition, a flowery "rosy" note was observed. These materials have been recommended repeatedly by aroma companies. However, the materials do not seem to be suitable for use with menhaden oil. The same observation was made with "violet" flavor.

Flavoring materials with "fruity" flavor properties.—Citral added a refreshing lemon odor and flavor to fish oil; but in addition, it is sharp in taste with the sharpness lingering in the aftertaste. Repeat tests with other concentrations confirmed that citral alone is too sharp. In combination with fruity flavoring compositions, it might help to introduce a refreshing note.

Melonal (2,6-dimethyl-5-hepten-1-al) contributed a fruity refreshing note, lingering in the aftertaste. In addition, it gave a greenness similar to that described by some panel members as "green-melony." Unfortunately, the sample turned rancid rapidly toward the end of the

5-day period of storage when melonal was added.

γ-Undecalactone (peach lactone), when used alone, is too artificial in odor and too sharp in flavor.

Lemon oil made the odor of the menhaden oil aromatic-fruity. It was retested with good results.

Lime oil gave a refreshing citrus taste to the menhaden oil, contributed some astringency, but it did not linger in the aftertaste. The aftertaste was more lemon-"candy"-like. (It was probably not pure lime oil; artificial flavoring material probably had been added, giving this "candy"-like flavor.)

Orange oil has good masking properties.

Blood Orange Flavor, Lemon Juice Flavor, Lemon Mint Flavor, Lime Mint Flavor, Orange Mint Flavor, Tetrarome Orange had good masking properties.

Imitation Boysenberry Flavor and Imitation Cranberry Flavor had masking properties for the odor rather than for the flavor. In addition, the flavor was quite artificial and unpleasant. The fruits that were imitated were not recognizable as such either in odor or in flavor. We tried to obtain a natural cranberry oil, but it is not available on the market.

Cherry Bouquet was outstanding in the masking properties of rancidity and oily feeling.

Wild Cherry Flavor also had very good masking properties.

Fruity Bouquet had very good masking properties; the flavor was fruity-candylike.

Imitation Melon Base, in alcoholic dilutions, gave a fruity-melon type of odor. In undiluted form, it smelled fruity and honey sweet. The oily feeling was well masked in the flavor with fruitiness and sweetness lingering in the aftertaste. After the oil was exposed for 1 day, there was in addition some lingering sharpness.

Imitation Oil Rum had good masking properties.

Flavoring materials with "butterlike" flavor properties.—Acetoin contributed a butterlike flavor and feeling, lingered in the aftertaste. The butter feeling is perceivable longer in the aftertaste than is the oily feeling. Unfortunately, the sample became rancid after only 3 days, indicating some prooxidant effect.

Butter Aroma showed good masking properties but only at the start of the test.

Flavoring materials with "sweet" flavor properties.—Benzaldehyde (bitter almond flavor) alone is not satisfactory. It has to be used in a flavoring complex such as Cherry Bouquet.

Ethyl phenyl acetate and Imitation Honey Flavor mask the oily feeling. In addition they contribute a honey flavor.

Vanillin was tried in different concentrations, but the results obtained were not satisfactory.

Flavoring materials with "spicy" flavor properties.—Anethol contributeed an anise flavor. It masked the oily feeling very well and changed it into a pleasant anise flavor (similar to that of cough drops flavored with anise oil or anethol).

Carvol and eugenol were not suitable. They added a sharp, somewhat burnt note to the menhaden oil.

Anise oil showed very good masking properties.

Bay oil, caraway oil, clove oil, and ginger oil were not suitable. They added a sharp, somewhat burnt note to the fish oil.

Cassia oil and cinammon oil were equally suitable.

Flavoring materials of the "wintergreen" group.—Ethyl salicylate, methyl salicylate, and root beer oil all masked the oily feeling very well.

Flavoring materials with "cooling" flavor properties.—Menthol, peppermint oil, and spearmint oil contributed initially only a bland taste and pleasant oily feeling to the menhaden oil. Samples with eucalyptol had a sharp note in flavor. All samples had a rancid flavor at the end of the 5-day storage test at room temperature.

Reduction in the List of Potential Masking Materials

The number of potential flavoring materials for masking the objectionable flavor components in the menhaden oil, based upon the preliminary screening tests, was too large to subject them all to a large-scale consumer-type test and therefore had to be decreased. The most promising flavoring materials for masking were:

Synthetics and isolates:
- Anethol
- Ethyl phenyl acetate
- Ethyl salicylate
- cis-hexen-3-ol-1
- Methyl salicylate

Essential oils:
- Anise oil, U.S.P.
- Cassia oil
- Cinnamon oil, U.S.P.
- Lemon oil
- Orange oil, U.S.P.
- Root beer oil

Imitation flavoring compositions:
- Blood Orange Flavor
- Butter Aroma
- Cherry Bouquet
- Fruity Bouquet
- Imitation Honey Flavor
- Imitation Lemon Juice Flavor #51.124A
- Lemon Mint Flavor
- Lime Mint Flavor
- Orange Mint Flavor
- Tetrarome Orange
- Imitation Oil Rum #017
- Imitation Oil Wild Cherry #12009
- Tutti Frutti Oil

Because using more than a dozen flavoring materials in the large-scale consumer tests was not desirable, we considered limiting further the number of flavoring materials for additional study. The results of this phase of the examination permitted us to eliminate additional materials, based upon the following observations.

Ethyl phenyl acetate was described as perfumelike by some consumers and was therefore disliked by them.

Ethyl salicylate was partly liked, partly disliked by different panel members. The scores for methyl salicylate were higher.

Cis-hexen-ol-1 tested in samples stored at 40° F and —20° F for 8 weeks did not result in observable rancidity. The samples still had a green-grassy character. The control sample stored at 40° F was rancid; the control sample stored at —20° F was almost odorless and was faint cucumber green in flavor.

Even though cis-hexen-3-ol-1 masked the oily feeling and rancidity, it was not liked by the panel.

Cassia oil and cinnamon oil have much the same flavor; cinnamon oil was therefore arbitrarily selected for further experiments.

To reduce the number of fruity flavors, we made a preliminary consumer test. From six flavors—Blood Orange, Lemon Juice, Lemon Mint, Lime Mint, Orange Mint, and Tetrarome Orange—Lemon Juice Flavor was chosen for further experiments.

Fruity Bouquet with its candy, fruitlike flavor was disliked by some panel members who described it as being perfumelike.

Cherry Bouquet not only was outstanding in the masking properties of rancidity and oily feeling but received the highest rating in the consumer test.

Only flavoring materials allowed by the U.S. Food and Drug Administration could be considered. The Cherry Bouquet and Fruity Bouquet are perfume compositions and are not permitted as food additives.

Butter Aroma was not satisfactory. As was already experienced with acetoin, samples flavored with a butter-aroma composition turned rancid faster in the storage test at 40° F than did the control. The 40° F sample was definitely rancid after 8 weeks of storage, although the samples held below freezing were not.

Imitation Honey Flavor was too artificial in flavor and therefore disliked by most of the panel.

Masking of Fish Oil with Pronounced Burnt Flavor

As was described in Materials, menhaden oil was refined both by the regular three-stage (clay bleaching, molecular distillation, silica gel)

process and by a modified two-step process in which the silica-gel procedure was eliminated but in which the collection of distillate during molecular distillation was restricted. Such a procedure greatly simplified refining but resulted in a menhaden-oil product containing considerable burnt-flavor component. Flavoring materials giving best results with the latter oil did not necessarily give optimum results with the silica gel refined oil.

Of the flavoring materials found to be most successful for the ordinarily refined procedure, those with a fruity-type flavor—especially Cherry Bouquet and Fruity Bouquet—masked the burnt flavor better than did such flavors as root beer oil, methyl salicylate, or anethol. None of these compounds was able to mask the burnt flavor completely when added to the freshly distilled oil. After 6 weeks of storage at 40° F (4° C) samples masked with Cherry Bouquet or Fruity Bouquet had no burnt flavor, but considerable remained when root beer oil or methyl salicylate was used, and an intermediate amount remained when anethol was used.

Flavoring materials having good masking properties and giving promise for good consumer acceptability were as follows: anethol, anise oil, cinnamon oil, lemon oil, Lemon Juice Flavor, methyl salicylate, orange oil, root beer oil, Imitation Oil Rum, Imitation Oil Wild Cherry, and Tutti Frutti Oil.

When considerable burnt flavor is present in menhaden oil, such as occurs when the two-stage modified refining process is used, none of the masking agents completely obscures this burnt flavor. Certain fruity flavors such as Cherry Bouquet or Fruity Bouquet are the most effective in masking this flavor, with methyl salicylate or root beer oil being less effective.

EXPERIMENT 2—CONSUMER-TYPE TESTS

The next stage of the investigation was to determine consumer hedonic rating for the several masking substances that had been found to be almost equally suitable for disguising the objectionable flavors developing in menhaden oil during storage.

MATERIALS

The same materials—both menhaden oil and flavor additives—were used in this phase.

The flavors used were those that in Experiment 1 had given the best masking results. Any flavors that were used in Experiment 1 and that were not approved for food additive use by the U.S. Food and Drug Administration were eliminated in this part of the work. Table 2 shows the identity and source of these substances.

TABLE 2.—Flavor components rated in the consumer test.

Flavor material	Suppliers[1]
Anethol, U.S.P.	1,4
Anise oil, U.S.P.	3
Cinnamon oil, U.S.P	3
Lemon oil, U.S.P.	4
Imitation Lemon Juice Flavor #51.124A	2
Methyl salicylate	1,4
Orange oil, U.S.P.	4
Root beer oil	3
Imitation Oil Rum #017	4
Imitation Oil Wild Cherry #12009	4
Tutti Frutti Oil	4

[1]
1 — Felton Chemicals Co., Inc., Brooklyn, N.Y.
2 — Firmenich, New York, N.Y.
3 — Florasynth Laboratories, Inc., New York, N.Y.
4 — Fritzche Brothers, Inc., New York, N.Y.

METHODS

A modified molecular distillation procedure was used. This procedure eliminated the necessity for carrying out the time-consuming treatment with silica gel. The oil was distilled only until the pot residue amounted to 10 % (as contrasted to 3 % in the usual methods). This decreased amount of distillation resulted in much less burnt flavor (although in considerably more burnt flavor than when a silica-gel treatment was used). The oil could be used, however, directly without silica-gel treatment when sufficiently effective masking agents were used.

Oils were stored at (1) 40° F (4° C) (2) at —20° F (—29° C) for 4 to 12 weeks.

The panel for the consumer-type test consisted of Bureau of Commercial Fisheries personnel who had no previous experience in taste testing. The 15 to 20 participants were asked to rate

Name .....

Date .....

Please indicate by check mark (✓) how much you like or dislike each sample and rank it in order of preference (1-best, 2-second best, etc.).

Hedonic Scale	Scale	Code	Code	Code
Like very much	1			
Like moderately	2			
Neither like nor dislike	3			
Dislike moderately	4			
Dislike very much	5			

Comments:

Too weak

Satisfactory

Too strong

Flavor recognized as:

Ranking order:

FIGURE 1.—Score sheet for the consumer test.

two to three flavored samples on a hedonic scale, using the score sheet shown in Figure 1.

Five ml of oil was rated by the panel 5 days a week for about 3 months. It was necessary to determine whether a flavor, liked initially, was still liked after continuous intake. Likes and dislikes were expressed on an hedonic scale of 5 points (Fig. 1).

RESULTS

Table 3 summarizes the consumer ratings over the period of test.

Some consumers were consistent in their rating throughout the entire period of the test. Other consumers were consistent only with some flavors but were inconsistent with others.

DISCUSSION

Table 3 shows that all of the tested flavors received high ratings (1 or 2) as main ratings by the consumer-type panel. It should be noted that these high ratings were not given by the consumer panel as a whole but rather by groups within the panel. It also should be noted that there is a split of like and dislike for the same flavor.

This split is understandable, and it may be due to different causes. Individuals with various background show widely different flavor preferences. For example, Europeans who are used to methyl salicylate being used primarily for flavoring medicinal products or in disinfectants would doubtlessly have rated this substance lower for fish-oil masking than did our American panel. In parts of Asia where anise seed

TABLE 3.—Consumer ratings for different flavored fish oil.

Flavoring material added to fish oil	Hedonic ratings[1]				
Anethol	1,	2,		(4)	
Anise oil	(1)	2,		(4)	
Cinnamon oil	1,	2,	(3)	4	
Lemon oil	(1)	2,	(3)	(4)	
Imitation Lemon Juice Flavor #51.124A	1,	2,		(4)	
Methyl salicylate	1,	2,	3,		(5)
Orange oil	(1)	2,	3,	(4)	
Root beer oil	1,	2,	(3)	(4)	
Imitation Oil Rum #017	(1)	2,	(3)	(4)	(5)
Imitation Oil Wild Cherry #12009	1,	(2)	3,	(4)	
Tutti Frutti Oil	1,	2,		(4)	

[1] Rating numbers not in parentheses were the ones given by most consumers, those in parentheses were the ones given by fewer members—for example, lemon oil was liked moderately (Rating 2) by most consumers, but some gave a higher rating (1) and some gave a lower rating (3) or (4).

is grown, frequently anise is disliked as a flavoring material. In addition to differences due to nationality, childhood experience has a definite influence on likes and dislikes. Orange oil is a good example. Panel members who had been forced during childhood to take orange-flavored cod-liver oil as a medicine objected to orange flavor. As can be observed in daily food intake where some persons prefer sweet foods and others prefer acid foods, there is likewise the same split of like and dislike with sweet flavoring materials (cinnamon oil and Tutti Frutti Oil) and with acid flavors (Lemon Juice Flavor).

Even though the consumer-type panel as a whole did not rank the flavors in a definite order of preference, a pronounced like for any of the offered flavors by smaller consumer groups is obvious. This finding should encourage trial of these fish-oil masking flavors with larger consumer groups.

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EARLY DEVELOPMENTAL STAGES OF THE BROWN SHRIMP, *Penaeus aztecus* IVES, REARED IN THE LABORATORY[1]

HARRY L. COOK[2] AND M. ALICE MURPHY[3]

ABSTRACT

The larval and first postlarval stages of the brown shrimp, *Penaeus aztecus* Ives, reared from eggs spawned in the laboratory, as well as the eggs themselves, are described and illustrated. The larvae and first postlarva are compared with those of the pink shrimp, *P. duorarum* Burkenroad, and white shrimp, *P. setiferus* (Linn.).

Commercial shrimp landings from Gulf of Mexico and South Atlantic waters of the United States are comprised mainly of three species: the brown shrimp, *Penaeus aztecus* Ives; the pink shrimp, *P. duorarum* Burkenroad; and the white shrimp, *P. setiferus* (Linn.). The Gulf coast shrimp fishery is the most valuable of domestic commercial fisheries, and in 1968 the value to fishermen for shrimp caught in the Gulf amounted to nearly $95 million. Because of their relative importance, these shrimps are being intensively studied by the National Marine Fisheries Service in an effort to understand more fully their biology and ecology.

Thirteen species of penaeid shrimp representing five genera inhabit the shallow nearshore waters of the northwestern Gulf. At the present time their larvae can only be identified to genus (Cook, 1966). Within genera the larvae are so similar morphologically that at any given stage the various species cannot yet be distinguished. Since adults of the three commercially important species often occur together and overlap in their spawning activity, there is also some intermingling of their planktonic larvae. To answer basic questions about larval distribution, growth, and survival of each species, accurate identification of larvae is essential.

The larval and early postlarval stages of the pink shrimp were described by Dobkin (1961), and those of the white shrimp by Pearson (1939) and Heegaard (1953). These shrimp have five nauplial, three protozoeal, three mysis, and several postlarval stages. Their morphological characteristics during these stages are so alike, however, that biologists still encounter difficulty in differentiating the two species. Since the early developmental stages of the brown shrimp have not been described, the purpose of this paper is to do so and at the same time compare them with corresponding stages of the pink and white shrimps.

METHODS AND MATERIALS

Larvae of the brown shrimp were first successfully cultured in the laboratory during the fall of 1963. Since that time, culture techniques have improved greatly. The methods used in our culture of larval penaeid shrimp were described by Cook and Murphy (1966), Cook (1969), and Cook and Murphy (1969).

Specimens of each stage were preserved for descriptive purposes. Drawings were made with the aid of a camera lucida. The figures of each substage are composite and represent an "average" larvae rather than any one individual. Also, with the exception of the nauplii, appendages on these figures are intended to indicate only relative size and position, not to show details of setation. The enlarged figures of

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mouth parts and other appendages were taken from two individuals representing each substage. Setules on the setae of the larvae were deliberately omitted, and nauplial appendages were rotated on their axes to avoid cluttering the figures and obscuring important diagnostic characters.

Abbreviations used in the text are: TL = total length, including rostrum when present but excluding caudal spines; W = body width at the point of greatest width; CL = carapace length, including the rostrum; and N = the number of specimens examined.

DESCRIPTION OF DEVELOPMENTAL STAGES

EGG AND HATCHING

(Fig. 1)

Diameter 0.26 mm

Viable eggs are round, golden brown, and translucent. As the nauplius develops, it fills the egg case and can be seen moving sporadically. At hatching, the egg case splits and the posterior portion of the nauplius protrudes. Then the nauplius, unmoving, appears to swell until it is forced from the shell, the entire process taking about 30 sec.

When first hatched, the nauplius rests almost motionless for 3 to 5 min. Although the appendages do not move during this time, spastic movement can be seen within the body near the base of each appendage. Suddenly the nauplius folds its appendages posteriorly along the ventral margins of the body, and, in one quick movement, sheds from its posterior end a loose-fitting exoskeleton and starts swimming actively. At first, the nauplius alternates 2- to 3-sec periods of swimming with resting periods of equal duration.

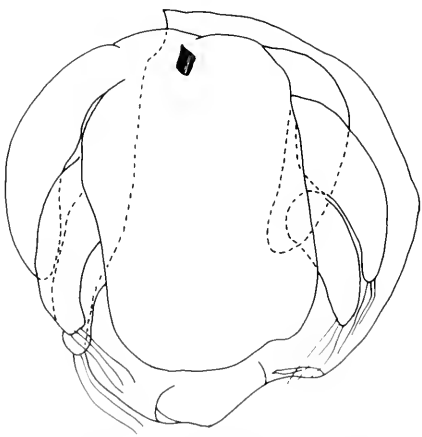

FIGURE 1.—Nauplius emerging from egg.

NAUPLIUS I

(Fig. 2)

Mean TL = 0.35 mm (0.32-0.38 mm)

Mean W = 0.19 mm (0.18-0.21 mm)

N = 55

The pyriform body is unsegmented and possesses a ventrally projecting labrum. An ocellus, which persists in subsequent nauplial substages, is present near the anterior end. The dorsal surface of the body is smooth except for a small median spine posteriorly (Fig. 2b). The posterior portion of the body is rounded and bears a single pair of caudal spines.

The body color in all nauplial substages is a

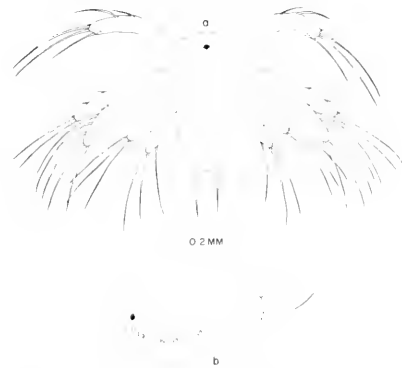

FIGURE 2.—Nauplius I: a, ventral view; b, lateral view.

golden brown, with the appendages tinged red apically. The translucent body appears filled with small granules that flow freely when the cuticle is ruptured.

Three pairs of appendages are present. The first antennae are uniramous and slightly less than three-quarters the length of the body. Each second antenna is biramous, its endopod slightly shorter than the exopod, and approximately three-quarters the body length. The mandibles are biramous and slightly less than one-half the body length.

In this substage all setae are smooth, but in subsequent substages, the longer ones are plumose.

Setation of appendages:

First antenna: Two short ventrolateral; two long terminal plus a small spike; one long dorsolateral.

Second antenna:

Endopod: Two short ventrolateral; two long terminal plus a small spike.

Exopod: Three long ventrolateral; two long terminal.

Mandible: Both branches bear three long setae.

NAUPLIUS II

(Fig. 3)

Mean TL = 0.39 mm (0.36-0.41 mm)
Mean W = 0.20 mm (0.20-0.21 mm)
N = 27

Body shape is similar to that of the first nauplius except that the posterior end is no longer rounded; the portion between the single pair of furcal spines has become straightened. The small dorsomedian spine near the posterior end (present in the first substage) is absent in this substage.

Setation of appendages:

First antenna: One short and one medium ventrolateral; one short, one medium, and one long terminal; one medium dorsolateral.

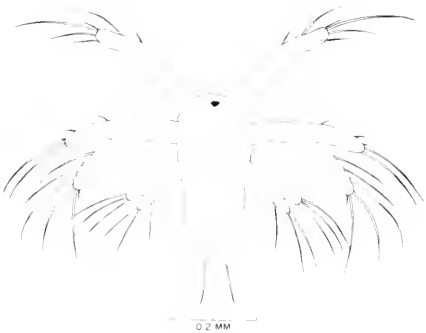

FIGURE 3.—Nauplius II, ventral view.

Second antenna:

Endopod: Two short ventrolateral; one small spike and two long terminal.

Exopod: Three long ventrolateral; two long and one short terminal.

Mandible: Same as Nauplius I.

NAUPLIUS III

(Fig. 4)

Mean TL = 0.40 mm (0.36-0.43 mm)
Mean W = 0.21 mm (0.20-0.23 mm)
N = 51

The posterior portion of the body is more elongate than in previous substages, but the shape remains essentially the same. The body is slightly depressed between the two developing furcal processes, each of which bears four spines. The small dorsomedian spine, absent in the second nauplius, reappears. The beginnings of ventral appendages can be seen as small indentations posterior to the labrum. The bases of the mandibles have become slightly swollen, and small frontal organs are present at the anterior end of the body.

Setation of appendages:

First antenna: One short and two medium ventrolateral; one short, one medium, and one

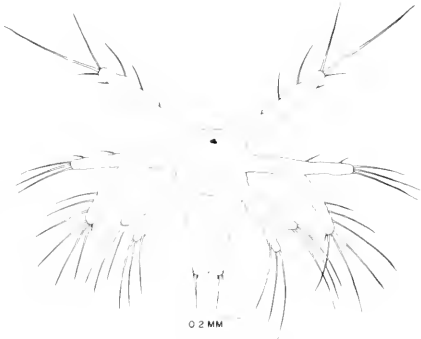

FIGURE 4.—Nauplius III, ventral view.

long terminal; one short dorsolateral.

Second antenna:

Endopod: Two short ventrolateral; three long terminal.

Exopod: Three long ventrolateral; three long and one short terminal.

Mandible: Same as Nauplius I.

NAUPLIUS IV

(Fig. 5)

Mean TL = 0.44 mm (0.41-0.47 mm)
Mean W = 0.21 mm (0.20-0.22 mm)
N = 35

The posterior portion of the body has become more slender and two definite rounded furcal processes are formed, each one with six spines. The small dorsomedian spine on the body is absent and does not reappear in later substages. Ventral appendages (first and second maxillae and first and second maxillipeds), still covered by the cuticle, are visible posterior to the mandibles. Frontal organs are present.

Setation of appendages:

First antenna: Same as Nauplius III.

Second antenna:

Endopod: Two short ventrolateral; one short and three long terminal.

Exopod: Four long ventrolateral; two long, one medium, and one short terminal.

Mandible: Same as Nauplius I.

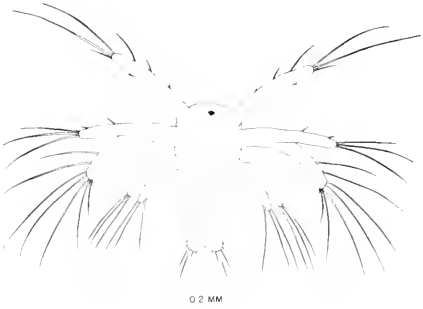

FIGURE 5.—Nauplius IV, ventral view.

NAUPLIUS V

(Fig. 6)

Mean TL = 0.50 mm (0.43-0.58 mm)
Mean W = 0.20 mm (0.18-0.22 mm)
N = 41

The body is further elongated and the furcal processes are more pronounced, each giving rise to seven spines. The maxillae and maxillipeds are now external and show more advanced development. The swelling at the base of the mandible is large and prominent and has a masticatory surface composed of several rows of small teeth. The endopod and the exopod of the mandible are frequently hollow and transparent. The outline of a developing carapace can be seen on the dorsal surface of the body, and frontal organs are present.

In living specimens, eyes and an anal canal are visible internally. In preserved specimens, the anal canal appears to open externally.

It was not possible to determine if the appendages were truly segmented. The appendages of some specimens appeared segmented, i.e., their surfaces possessed annular indentations; those of others did not.

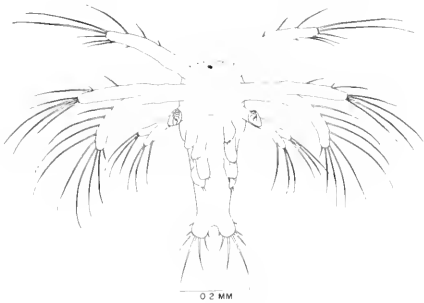

FIGURE 6.—Nauplius V, ventral view.

Setation of appendages:

First antenna:	Two short and one medium ventrolateral; two long and one medium terminal; two medium and one short dorsolateral.
Second antenna:	
Endopod:	Two short and one medium ventrolateral; one medium and three long terminal.
Exopod:	Five long ventrolateral; three long and one short terminal.
Mandible:	Same as Nauplius I.

PROTOZOEA I

(Fig. 7)

Mean TL = 0.96 mm (0.89-1.21 mm)
Mean CL = 0.45 mm (0.40-0.49 mm)
N = 40

With the molt from Nauplius V to Protozoea I, the larvae change radically. A large, loose-fitting carapace covers the anterior portion of the body. The narrow posterior portion is divided into a six-segmented thorax and an unsegmented abdomen. The masticatory surface of the mandible has become greatly enlarged, and the endopod and exopod have been lost. The maxillae and maxillipeds are large and functional.

The carapace is rounded with a median notch at the anterior end; two rounded frontal organs are the only protuberances on it. An ocellus, which persists in subsequent protozoeal substages, is present between a pair of compound eyes covered by the carapace. The labrum is smaller than in the preceding stage and has a short spine on its anterior margin. Two lobes of the labium, with short bristles on their inner margins, are posterior to the labrum. The mandibles curve inward and several of their teeth can be seen between the labrum and labium.

The first antenna, which is about equal in length to the endopod plus the protopod of the second antenna, is composed of three major segments. The basal segment is divided into five subsegments and bears one short seta. The second segment possesses three setae, two ventrolateral, and one posterolateral. The distal segment has three terminal and three subterminal setae.

The second antenna consists of a protopod of three segments, an endopod of two segments, and an exopod of 10 segments. The endopod bears one seta at the juncture with the protopod, another on the first segment, two at the juncture of the first and second segments, and five terminal setae on the distal segment. The exopod has eight setae on its ventrolateral and two on its dorsolateral margins, as well as three terminal setae.

The mandible has lost its exopod and endopod. The masticatory surface now faces medially and has several rings of teeth.

The first maxilla is composed of an unsegmented protopod, an endopod of three segments, and a small knoblike exopod. The protopod consists of two large lobes, each bearing several stout, toothed spines. The first segment of the endopod possesses two or three setae; the second, two; and the distal, five. The exopod bears four setae.

The second maxilla is about the same size as the first. It has an unsegmented protopod, an endopod of four segments, and a knoblike exopod. The protopod has five lobes on its ventral margin; the basal lobe bears approximately eight setae, the remainder three to six. The first three segments of the endopod each

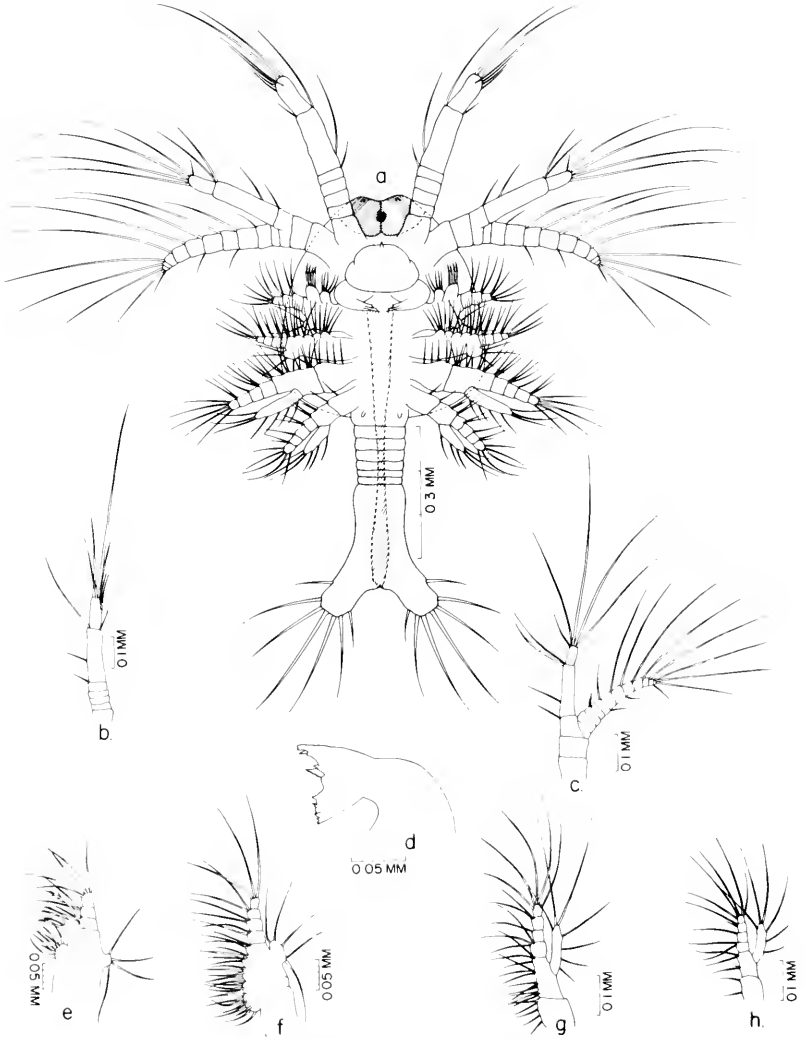

FIGURE 7.—Protozoea I: a, ventral view; b, antenna I; c, antenna II; d, mandible; e, maxilla I; f, maxilla II; g, maxilliped I; h, maxilliped II.

have two setae, and the fourth, three. The exopod has five setae.

The first maxilliped is biramous and longer than the maxillae. It has a protopod of two segments, an endopod of four segments, and an unsegmented exopod. The protopod bears about 17 setae. The basal segment of the endopod possesses three setae; the second, one or two; the third, two; and the terminal, five. The exopod has four lateral and three terminal setae.

The second maxilliped, though smaller, is almost identical to the first. The protopod bears six setae. The endopod is composed of four segments, the first and third giving rise to two setae; the second, one or two; the fourth, five. The exopod has three lateral and three terminal setae.

The third maxilliped is present only as a small bud.

The caudal furcae each retain seven spines and are separated by a well-defined anal opening. The digestive tract is visible posterior to the labrum.

The body is colorless and almost transparent with the exception of two red spots, one on each side of the anal opening.

PROTOZOEA II

(Fig. 8)

Mean TL = 1.71 mm (1.28-2.01 mm)
Mean CL = 0.80 mm (0.72-0.87 mm)
N = 25

The most apparent modification from the preceding substage is the presence of stalked compound eyes. Additional features that characterize this substage are a ventrally projecting rostrum, a pair of bifurcate supraorbital spines, and a segmented abdomen.

Frontal organs are absent in this and later stages.

Segmentation of the appendages remains the same as described for the first protozoea. The only changes in setation are on the first antenna. Several setules are added near the posterolateral seta on the second segment, and an additional terminal seta is found on the last segment. Rudiments of the third maxilliped and five pairs of pereiopods are present.

The abdomen is divided into six segments, the telson not being separated from the sixth. The number of furcal spines remains constant at seven pairs.

PROTOZOEA III

(Fig. 9)

Mean TL = 2.59 mm (2.40-2.59 mm)
Mean CL = 1.06 mm (0.93-1.40 mm)
N = 15

The principal differences between this substage and the preceding one are the presence of biramous uropods and spines on the abdominal segments.

The carapace is close fitting and covers all but the last three thoracic somites. The supraorbital spines are no longer bifurcate.

The five subsegments comprising the basal section of the first antenna in the preceding protozoeal substages have fused into a single unit. The ventrolateral seta that originated from the middle of the second segment has been lost, and a similar one is present on the distal margin. The second antenna, mandible, and maxillae remain essentially the same as in the preceding substage. Two setae have been added to the exopod of the first maxilliped. The first segment of the endopod and the exopod of the second maxilliped have gained a seta. Although the third maxilliped and five pereiopods have developed further and are now biramous, they remain functionless.

The abdomen is divided into six segments, the telson being distinct from the sixth segment. The sixth segment is about three-fourths the length of the preceding five combined. Each of the first five segments has a dorsomedian spine on its posterior margin. The fifth segment also possesses a pair of midlateral spines, and the sixth somite has paired midlateral and ventrolateral spines.

A pair of biramous uropods are present, originating from the ventroanterior margin of the telson. The exopod, slightly longer than the endopod, bears five or six setae at its apex.

An additional pair of caudal spines have been added medially on the telson, making a total of eight pairs.

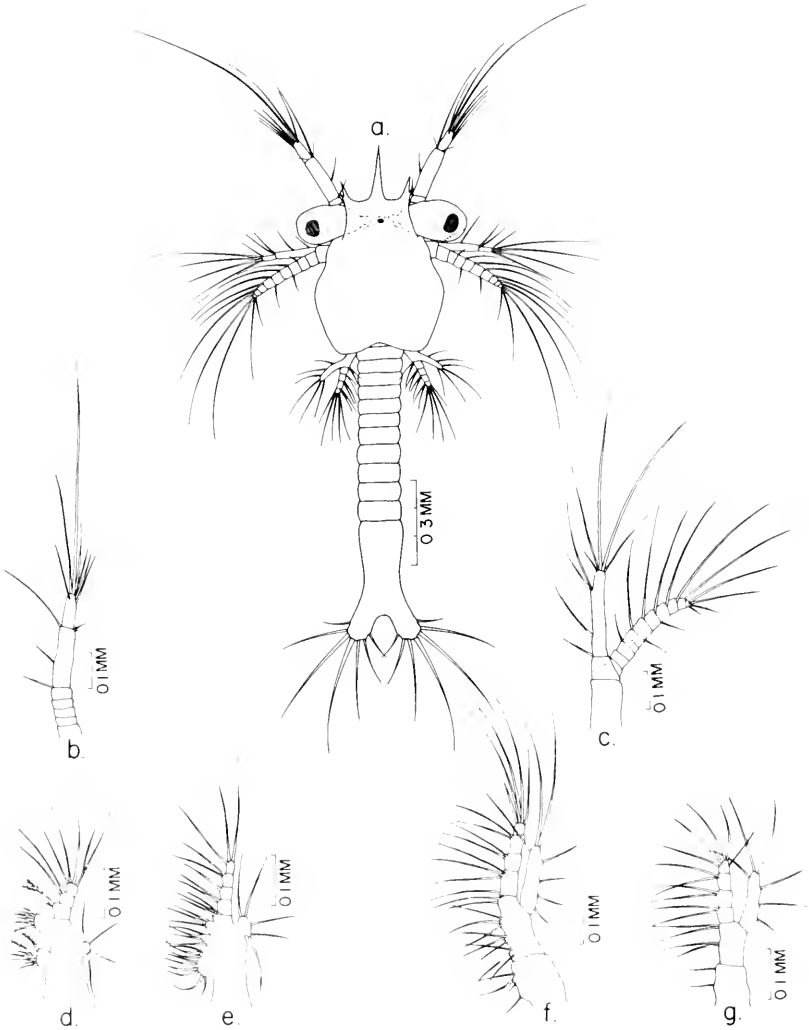

FIGURE 8.—Protozoea II: a, dorsal view; b, antenna I; c, antenna II; d, maxilla I; e, maxilla II; f, maxilliped I; g, maxilliped II.

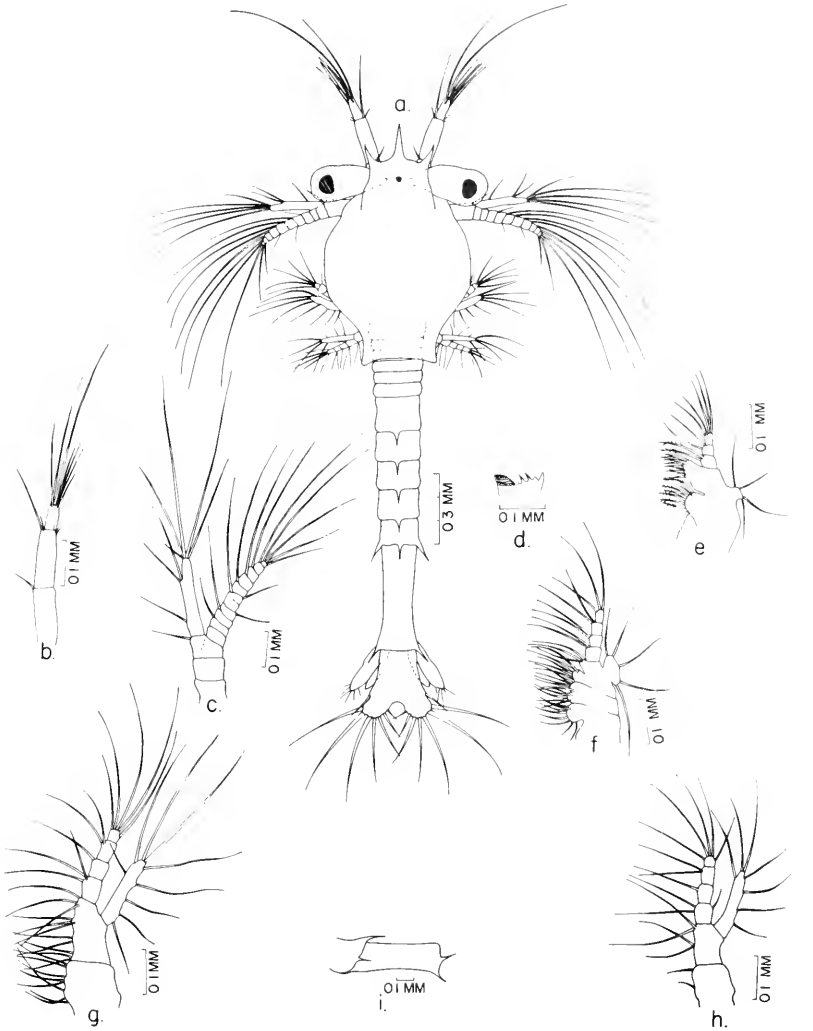

FIGURE 9.—Protozoea III: a, dorsal view; b, antenna I; c, antenna II; d, teeth of mandible; e, maxilla I; f, maxilla II; g, maxilliped I; h, maxilliped II; i, abdominal segments V and VI.

MYSIS I
(Fig. 10)
Mean TL = 3.3 mm (3.2-3.5 mm)
Mean CL = 1.2 mm (1.1-1.3 mm)
N = 18

With the molt from the third protozoeal to the first mysis substage, the larvae undergo another radical change and assume a more shrimplike appearance. The most apparent change is the development of functional pereiopods with long brushlike exopods. The antennae also undergo considerable change, with the exopods of the second antennae becoming modified to form flattened antennal blades.

The carapace fits the body more closely than in preceding stages and covers all but the last two thoracic somites. The rostrum is not depressed as in the protozoeal substages, but protrudes forward on a horizontal plane. Supraorbital spines are still present although reduced in size. A small spine now occurs on the anteroventral corners of the carapace. In addition, a pair of hepatic spines have been added to the carapace, one spine on each side originating from a point located approximately one-sixth the carapace length from the anterior margin.

An ocellus is present in this and subsequent mysis substages.

The first antenna is composed of three segments, the first being 1½ times the length of the second and third combined. Numerous setae now occur along the appendage, and several arise at the apex of each segment. In addition, a prominent ventromedian spine is present on the first segment. The distal segment gives rise to two unsegmented branches, the external one bearing six or seven setae and being twice as long as the internal one, which bears two terminal setae.

The second antenna consists of a protopod of two segments, an unsegmented endopod with three lateral and three terminal setae, and a flattened, unsegmented, bladelike exopod with a single lateral seta and 11 setae along the medial and terminal margins.

The mandible and maxillae remain essentially the same as in the preceding substage except that the exopod of the second maxillae has become enlarged and bears 10 setae. The first and second segments of the endopod of the first and second maxillipeds have acquired an additional seta. The exopod of the first maxilliped has gained a seta and that of the second has lost three. The third maxilliped has evolved further and is now longer than the first two. It has a two-segmented protopod that bears two setae. The first segment of the five making up the endopod possesses two setae; the second, one; the third, none; the fourth, three; and the fifth, five. The unsegmented exopod has five or six terminal and subterminal setae.

The five pairs of pereiopods have undergone considerable enlargement and their exopods serve as the principal swimming organs during the mysis stage. The endopods of the first three pairs are modified into rudimentary chelae that have four or five terminal setae. The first pereiopod consists of a protopod of two segments, an unsegmented endopod, and an exopod which bears five or six terminal and subterminal setae. The last four pereiopods were not examined in detail.

The dorsomedian spines of the first two abdominal segments have been lost while those on the third, fourth, and fifth segments are still prominent. The fifth segment retains a pair of midlateral spines. A dorsomedian and a ventromedian spine are present on the sixth segment in addition to the paired midlateral and ventrolateral spines found in the preceding substage. Anlages of the pleopods can be seen on the ventral surface of the first five abdominal segments.

The uropod has developed an unsegmented protopod that possesses a large posteroventral spine and a smaller posterolateral spine. The endopod carries 11 setae on its medial and terminal borders, while the exopod, which has about 13 setae on its medial and terminal margins, has, in addition, a prominent spine on its posterolateral edge.

The telson is cleft terminally and bears seven pairs of terminal and one pair of lateral spines.

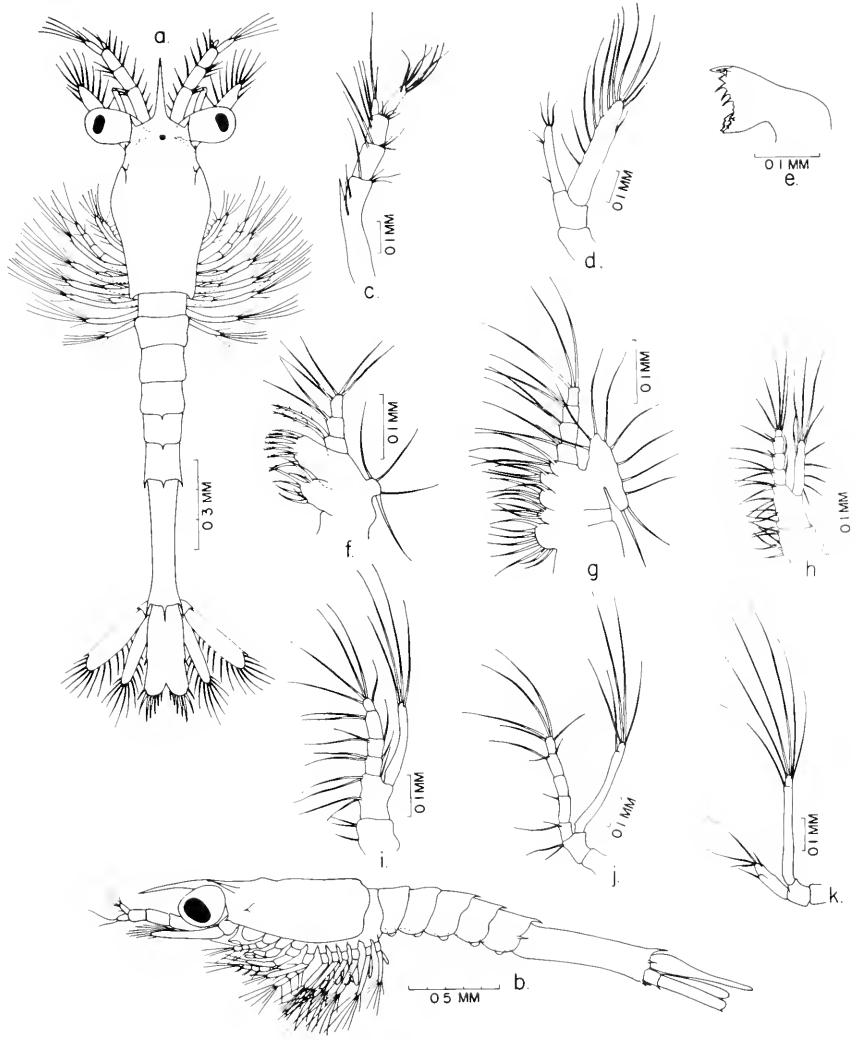

FIGURE 10.—Mysis I: a, dorsal view; b, lateral view; c, antenna I; d, antenna II; e, mandible; f, maxilla I; g, maxilla II; h, maxilliped I; i, maxilliped II; j, maxilliped III; k, pereiopod I.

MYSIS II

(Fig. 11)

Mean TL = 3.8 mm (3.3-4.2 mm)
Mean CL = 1.3 mm (1.2-1.4 mm)
N = 11

This substage can be distinguished from the first mysis by the presence of unsegmented pleopods and a spine on the antennal blade.

The addition of a dorsal spine on the rostrum is the only change in armature of the carapace which now covers the entire thorax.

The terminal branches of the first antenna are almost equal in length. A developing statocyst appears as a slight bulge near the base of the appendage.

Setation on the endopod of the second antenna has been reduced to a single terminal seta. The number of setae on the exopod has increased to 18, and they occur along the medial border and around the tip to the point of insertion of a subterminal spine on the lateral margin. A spine has been added to the terminus of the protopod.

The mandible has developed a small unsegmented palp.

The exopod of the first maxilla is no longer present; that of the second maxilla has increased in size and now bears 13 setae. A seta now arises from the protopod of the first maxilliped at a point between the insertion of the endopod and exopod. In addition to a seta on the distal segment, the endopod of the second maxilliped gains a fifth segment that does not possess setae. The protopods of the third maxilliped and first pereiopod have gained a seta. A single seta on the basal segment is the only one present on the three segments that have been added to the endopod of the first pereiopod. Rudiments of gills are present as small protuberances on the bases of the protopods of the maxillipeds and first pereiopods.

The armature of the abdomen and uropods is unchanged from the preceding substage. Rudimentary, unjointed pleopods are present on the ventral surface of the first five abdominal segments.

The telson has six pairs of terminal and two pairs of lateral spines.

MYSIS III

(Fig. 12)

Mean TL = 4.3 mm (3.9-4.7 mm)
Mean CL = 1.4 mm (1.3-1.5 mm)
N = 21

In this substage, the pleopods and endopod of the second antenna are composed of two segments. These characters serve to differentiate third mysis from preceding substages.

Spination of the carapace remains essentially the same as in Mysis II, although a second dorsal spine on the rostrum was found in approximately one-half of the preserved specimens.

The outer branch arising from the distal segment of the first antenna is longer than the inner branch and is composed of two segments. A lateral seta has been added to the endopod of the second antenna which is now also composed of two segments; the exopod likewise possesses one more seta.

The mandibular palp is slightly longer and has a weak apical seta.

The first maxilla and first maxilliped remain unchanged. The exopod of the second maxilla has become elongate and has 18 setae, while those of the second and third maxillipeds and first pereiopod have two segments. The fourth segment of the endopod of the second maxilliped has gained one seta, as has the exopod. One seta has been added to the second segment of the endopod of the third maxilliped and two to the third; the fourth has lost one. The endopod of the first pereiopod is composed of five segments, with the fourth forming the propodus of the chela and the fifth the dactylus; the second segment has gained one seta, and the third, two. The gills on the maxillipeds and first pereiopod have enlarged.

The only change of consequence in the posterior portion of the body involves the pleopods, which are now composed of two segments and bear two or three terminal setae.

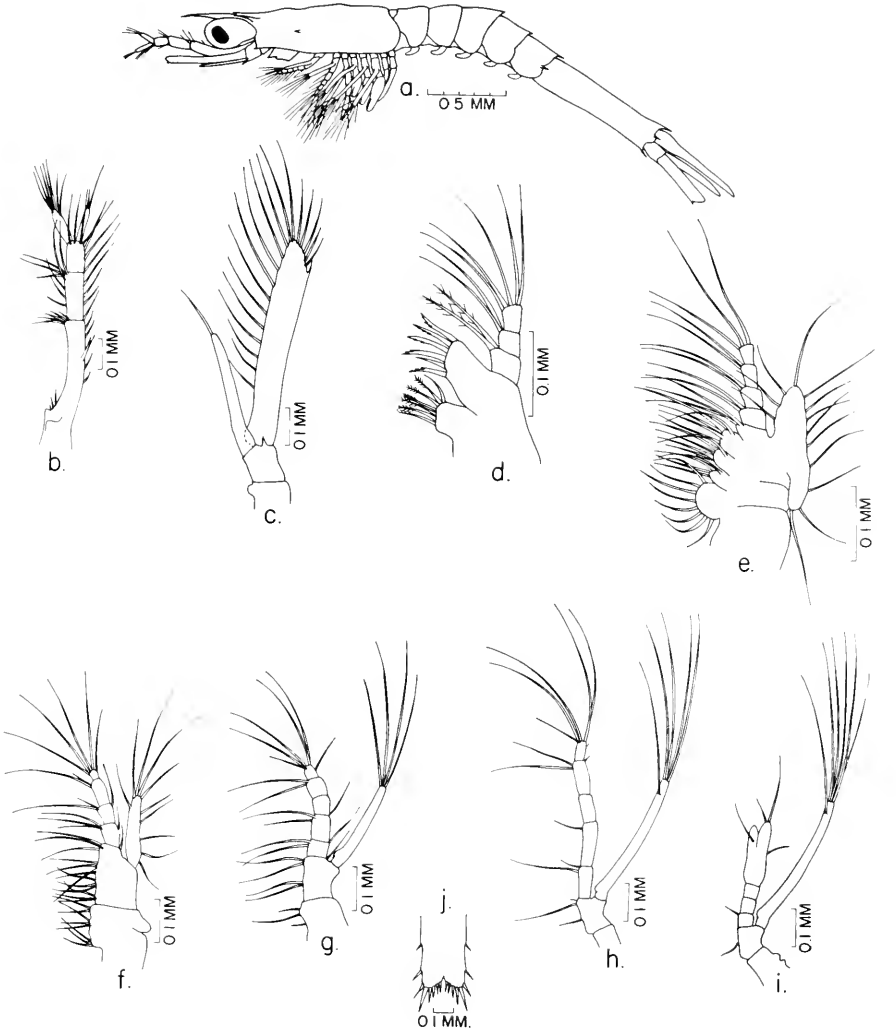

FIGURE 11.—Mysis II: a, lateral view; b, antenna I; c, antenna II; d, maxilla I; e, maxilla II; f, maxilliped I; g, maxilliped II; h, maxilliped III; i, pereiopod I; j. tip of telson.

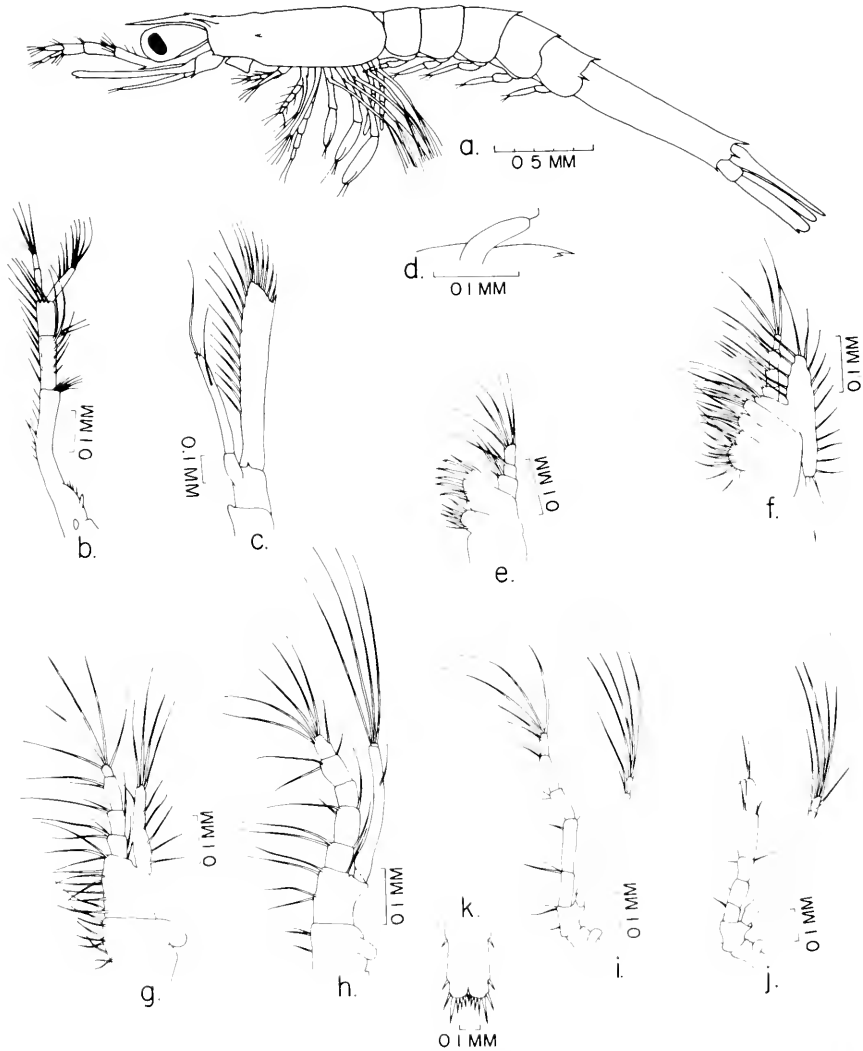

FIGURE 12.—Mysis III: a, lateral view; b, antenna I; c, antenna II; d, mandibular palp; e, maxilla I; f, maxilla II; g, maxilliped I; h, maxilliped II; i, maxilliped III; j, pereiopod I; k, tip of telson.

POSTLARVA I

(Fig. 13)

Mean TL = 4.6 mm (4.2-5.0 mm)
Mean CL = 1.5 mm (1.4-1.6 mm)
N = 15

No drastic changes in morphology are associated with the molt from the third mysis to the first postlarval stage. The pleopods, now well developed and setose, are the principal swimming organs. There is usually a reduction in the size of the exopods of the pereiopods, which, if present, are only vestigial.

The carapace is much the same as in the third mysis. The rostrum bears one or two spines and extends slightly beyond the distal border of the eye. The small spine present on the anteroventral corners of the carapace in the preceding substage is absent. The supraorbital spines are now minute or absent. A pair of hepatic spines and ocelli are present.

The inner branch of the first antenna is composed of two segments, and the outer, three. The statocyst at the base of the first antenna is fully developed. The endopod of the second antenna is composed of five or six frequently indistinct segments. The exopod bears 23 setae.

The mandibular palp is two-segmented and possesses five setae.

The endopods of the first and second maxillae have been reduced greatly and are now unsegmented and usually without setae. Setation of the protopod of the second maxilla has been reduced while its exopod has become enlarged and now bears 21 setae.

The first maxilliped retains only rudiments of its endopod and exopod. The second and third maxillipeds and the first pereiopod have lost all but a vestige of their exopods. The endopod of the second maxilliped has become recurved, and its setation has changed greatly: the first segment has three setae; the second, four; the third, one; the fourth, five; and the fifth, six. The second and third segments of the endopod of the third maxilliped have each gained one seta, the fourth, three; the number of setae on the terminal segment varies from three to six. The dactyl of the first pereiopod now possesses several small teeth and short bristles terminally.

Each pleopod is composed of two segments, the distal one bearing about 10 setae.

The presence of dorsomedian spines on the third, fourth, and fifth abdominal segments is variable. Such spines may be absent or present on one or more of the segments. The midlateral spines have been lost from the fifth and sixth segments. The sixth segment retains a dorsomedian and paired ventrolateral spines.

The telson, which is now only faintly cleft, bears five pairs of terminal and three pairs of lateral spines.

COMPARISON WITH PINK AND WHITE SHRIMP

During the fall of 1964, pink shrimp hatched from eggs spawned in the laboratory were reared to postlarvae. White shrimp were reared during the summer of 1966. Examination of these larvae showed them to be identical to brown shrimp larvae in setation and other major morphological characteristics. Various parts of the body were measured to determine if body proportions differed between the species; the results proved inconclusive.

Dobkin (1961) described the larval development of the pink shrimp but was absolutely certain only of the identity of the nauplial and first protozoeal substages which he obtained from eggs hatched in the laboratory. Descriptions of the more advanced stages were based on specimens sorted from plankton samples. When the pink shrimp we had reared were compared with the specimens described by Dobkin, several differences were noted. These are listed in Table 1.

Pearson (1939) and Heegaard (1953) described the larval development of the white shrimp from material taken in plankton tows. Comparison of these descriptions and our specimens was not attempted because we feel both Pearson's and Heegaard's descriptions of the later stages are not detailed enough for itemized comparison. In addition, Heegaard's editors felt that his figures of the first and second protozoeae were not referable to *P. setiferus*, but should be attributed to *Trachypenaeus*, *Sicyonia*, or *Xiphopenaeus*. From material at our disposal it

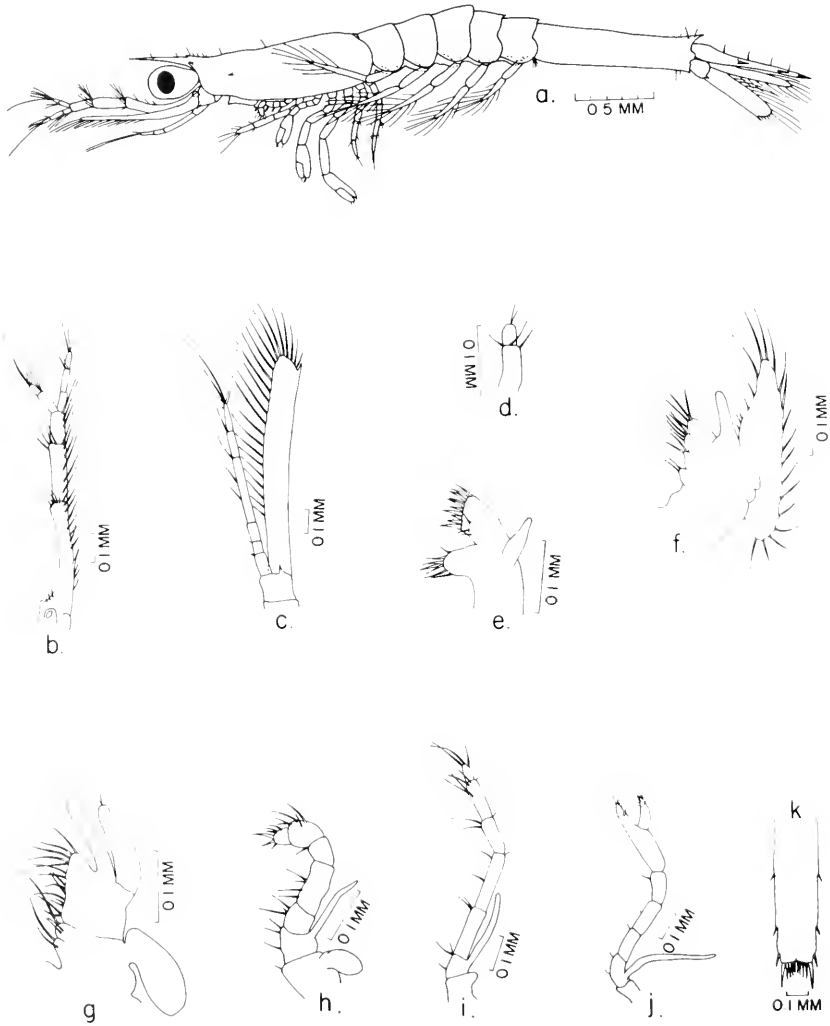

FIGURE 13.—Postlarva I: a, lateral view; b, antenna I; c, antenna II; d, mandibular palp; e, maxilla I; f, maxilla II; g, maxilliped I; h, maxilliped II; i, maxilliped III; j, pereiopod I; k, tip of telson.

TABLE 1.—Comparison between published description of *P. duorarum* and specimens examined by authors

Substage and body part	Dobkin (1961)	Cook and Murphy
Nauplius III		
Antenna I	No mention of posterolateral seta.	A minute posterolateral spine may be present.
Caudal spines	Three pairs.	Three or four pairs.
Nauplius IV		
Antenna I	Two anterolateral and no posterolateral spines.	Two or three anterolateral spines. Minute posterolateral spine may be present.
Caudal spines	Five pairs.	Six pairs.
Nauplius V		
Antenna I	Two posterolateral setae.	Two or three posterolateral spines.
Protozoea I		
Antenna I	Five terminal and subterminal setae. No posterolateral seta on middle segment.	Six terminal and subterminal setae. Middle segment with short posterolateral seta.
Protozoea II and III		
Antenna I	Five terminal and subterminal setae.	Seven terminal and subterminal setae.
Mysis		
Sixth abdominal segment	Two pairs of posterolateral spines.	One pair of posterolateral spines.
Protopod of uropad	Three spines on distal margin.	Two spines on distal margin.

would seem that the editors were correct and that the figures presented are species of *Trachypenaeus*.

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PROTEIN AUTOLYSIS RATES AT VARIOUS pH'S AND TEMPERATURES IN HAKE, *Merluccius productus*, AND PACIFIC HERRING, *Clupea harengus pallasi*, AND THEIR EFFECT ON YIELD IN THE PREPARATION OF FISH PROTEIN CONCENTRATE

BARBARA KOURY, JOHN SPINELLI, AND DAVE WIEG[1]

ABSTRACT

The rate of protein autolysis at temperatures ranging from 30° to 80° C and at pH's ranging from 3.0 to 7.0 were determined on hake and Pacific herring. Autolysis rates were generally greatest at acidic pH's and began to decrease after temperatures exceeded 50° C. Autolysis rates were much greater in hake than in Pacific herring. The yield of fish protein concentrate prepared from hake showed a close inverse correlation to the degree of autolysis.

The production of fish protein concentrate (FPC) requires a process that efficiently removes oil and water from the fish and provides high yields of protein. Although FPC can be prepared by several different methods (Knobl, 1967), the most effective procedures developed to date are based on systems in which comminuted fish is successively extracted with a suitable solvent system.

Dambergs (1969), in studying the extracting efficiency of isopropyl alcohol (IPA)-water mixtures in producing FPC from herring, found that low molecular weight compounds were readily removed with these solvent systems. Fish flesh and viscera contain highly active catheptic enzymes which utilize fish tissue as a substrate, forming low molecular weight protein degradation products (Siebert, 1962; Ting, Montgomery, and Anglemeier, 1968). Normal autolysis of fish tissue during storage has been related to decreased yields in FPC production. Dubrow, Brown, Pariser, Miller, Sidwell, and Ambrose (1971) found that when ice-stored fish was used to make FPC, the yield was significantly lower than the yield obtained when FPC was prepared from freshly caught fish. In another study by Dubrow and Hammerle (1969), in which FPC was prepared from samples of comminuted fish held in 91 % IPA for various lengths of time, similar results were obtained.

Preliminary studies in this laboratory, on the development of an aqueous process for FPC production, indicated that under certain conditions significant protein losses may also occur during actual processing procedures as a result of enzymatic hydrolysis.

In solvent extraction procedures for FPC production, the yield of product (excluding physical losses) is dependent upon the amount of proteinaceous material soluble in the extracting solution. While losses can be controlled to some extent by the choice of solvent systems, the possibility of losses due to enzymatic degradation of protein must also be considered.

The purposes of this study were as follows:

1. To determine the effect of pH, time, and temperature on the rate of protein autolysis in two species of fish that are considered for use in the production of FPC.

2. To determine the effect of autolytic activity on FPC yields.

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PROTEOLYTIC ENZYME ACTIVITY IN WHOLE AND EVISCERATED FISH

Presented in this experiment are data on the effect of time, temperature, and pH on the rate of proteolytic enzyme activity in samples of whole and eviscerated comminuted fish.

MATERIALS AND METHODS

Fish

Hake, *Merluccius productus*, and Pacific herring, *Clupea harengus pallasi*, were used in this study. Hake samples were obtained from both coastal waters (outside hake) and Puget Sound (inside hake). Herring were obtained from Bellingham Bay. All samples were obtained fresh and were immediately frozen and held at —20° C.

Preparation of Samples

To insure sample homogeneity, lots of fish were partially thawed and then ground in a Hobart grinder[4] through a $\frac{1}{8}$-inch plate. The ground fish was mixed well and divided into portions of approximately 50 g each. These were frozen to —40° C in a plate freezer and stored at —20° C.

Sufficient comminuted fish was prepared to permit running all pH and temperature variables on subsamples from the same lot. Samples of herring and outside hake were prepared from both whole and eviscerated fish. With inside hake, autolytic rates were measured only on whole fish.

METHODS

Fifty grams of comminuted fish were allowed to thaw at room temperature and then were mixed well with 100 ml of H_2O to form a slurry. The pH of the slurry was adjusted to the desired level with 0.1 M HCl and then sufficient water was added to give a final volume of 250 ml. After dilution, the sample was placed in a water bath.

Aliquots of the slurry were taken (1) immediately after the final dilution was made, (2) when the sample reached the desired temperature, and (3) at intervals of 10, 20, 30, and 60 minutes after the sample reached the desired temperature. These aliquots were mixed immediately with an equal volume of 10% TCA, allowed to stand for 30 min, and then filtered. The nonprotein nitrogen (NPN) content of the filtrates was determined by the procedure of Lowry, Rosebrough, Farr, and Randall (1951) and is presented in terms of optical density (OD) measurements at 500 mμ.

Rates of proteolytic enzyme activity at pH 6.0, 5.0, 4.5, 4.0, and 3.0 were measured at temperatures of 30°, 40°, 50°, 60°, 70°, and 80° C. Controls consisted of samples in which no pH adjustment had been made.

RESULTS AND DISCUSSION

The results of the above experiments are shown in Figures 1 through 5.

In whole herring (Fig. 1), no significant degree of proteolytic enzyme activity was observed at pH levels higher than pH 5.0 over the temperature range studied. At pH 4.0-4.5, maximum activity was found at 40° C, and at pH 3.0, maximum activity was found at 30° C.

Under these experimental conditions, only negligible proteolytic enzyme activity was observed in eviscerated herring samples (Fig. 2) indicating that in herring proteolytic enzymes are essentially of visceral origin.

Figures 3 and 4 show the results of the experiments using whole inside and outside hake. At 30° C, the rate of proteolytic activity in both control samples was negligible. As the pH was lowered, the rate of activity increased. At 40° C, the rate increased at all pH levels, and these rates remained elevated until the temperature exceeded 60° C. In these two samples, differences were observed in the pH of optimum activity at various temperatures. These variations were attributed to differences in the age, feeding habits, etc., of the two populations.

The effects of pH and temperature on the rate of enzyme activity in eviscerated outside hake

[4] The use of trade names is merely to facilitate descriptions; no endorsement is implied.

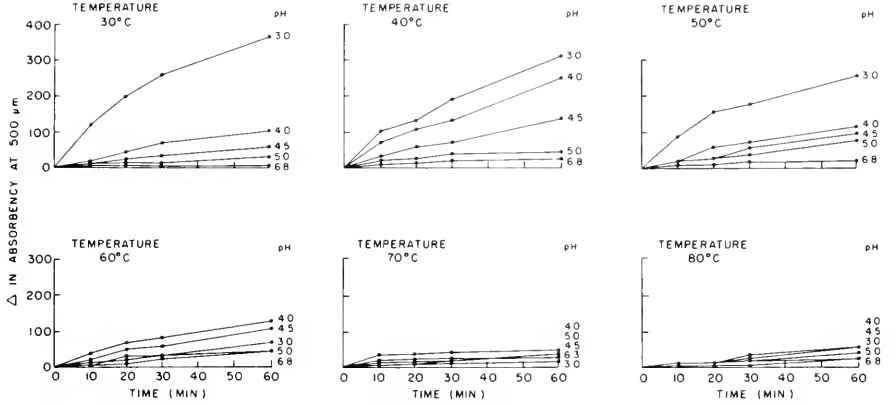

FIGURE 1.—Protein autolysis rates of whole herring homogenates held at various pH's and temperatures.

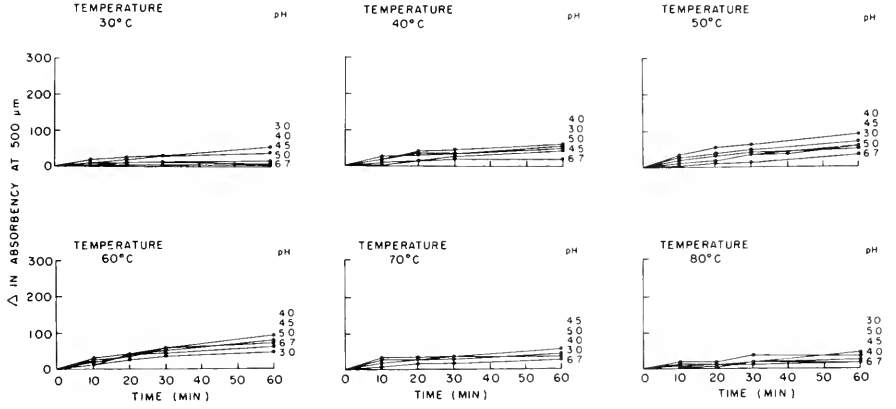

FIGURE 2.—Protein autolysis rates of eviscerated herring homogenates held at various pH's and temperatures.

are shown in Figure 5. The pattern is similar to that of whole hake, both in magnitude of activity and the effects of pH and temperature. Previous investigations in this laboratory (Dassow, Patashnik, and Koury, 1970) have shown that hake is often infected with the parasite, myxosporidian. The degree of this infestation is much greater in the outside than in the inside hake (Patashnik, personal communication, 1970). These investigators reported that infested hake autolyze rapidly during storage, making them unsuitable for processing into blocks or portions. No detailed study relating to parasites was made in this study, but the similar autolytic rates between whole and eviscerated outside hake shows that the main source of pro-

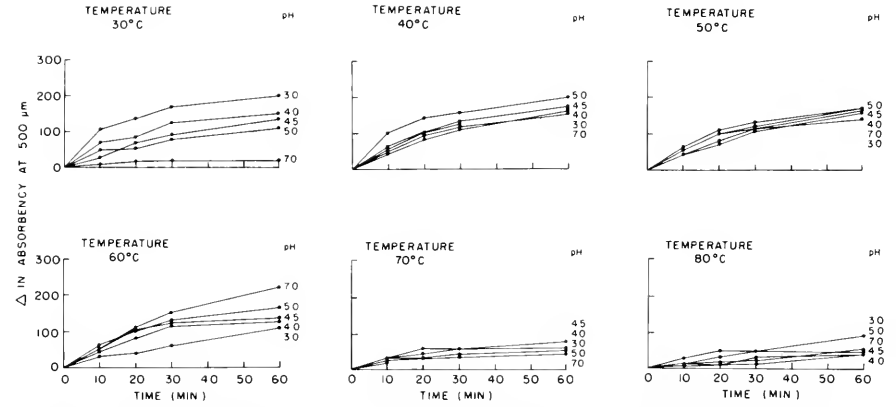

FIGURE 3.—Protein autolysis rates of whole hake (outside) homogenates held at various pH's and temperatures.

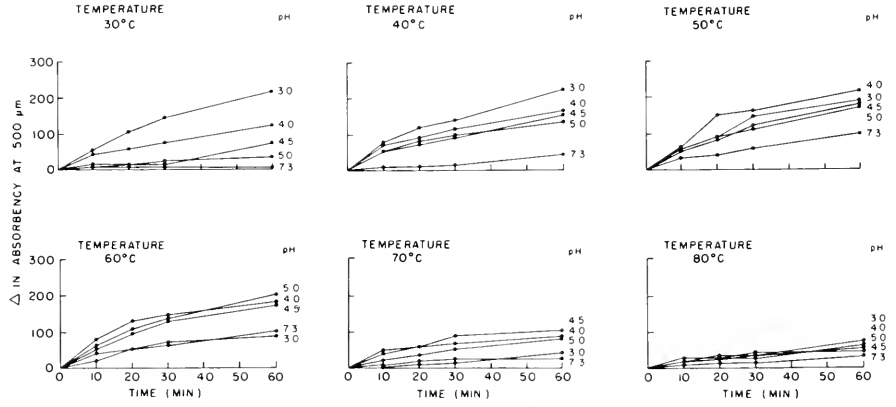

FIGURE 4.—Protein autolysis rates of whole hake (inside) homogenates held at various pH's and temperatures.

teolytic enzyme in the outside hake taken for this study resides in the tissue.

EFFECT OF AUTOLYTIC ACTIVITY ON YIELD OF FPC

MATERIALS AND METHODS

Whole inside hake were used throughout this experiment. For the preparation of FPC, whole hake was comminuted by passing through a Hobart grinder (equipped with a $\frac{1}{8}$-inch plate). Aliquots of the comminuted fish were acidified with 0.1 M HCl to pH 5.5 and 4.5. The original pH of the fish was 6.9. The treated fish was allowed to autolyze at 50° C for 0, 20, 40, and 60 min. After each time interval, FPC was prepared by isopropanol (IPA) extraction as follows: The fish was successively extracted (4

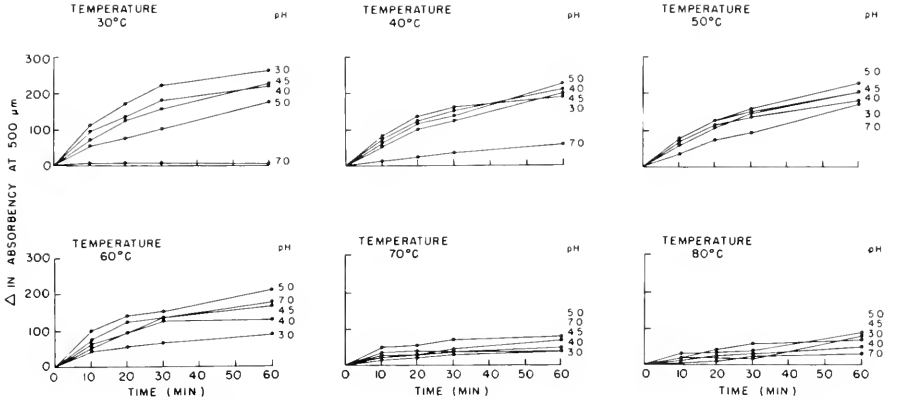

FIGURE 5.—Protein autolysis rates of eviscerated hake (inside) homogenates held at various pH's and temperatures.

times) with azeotropic IPA at a ratio of 2 parts IPA to 1 part fish. The first extraction was carried out at ambient temperature, and the final three extractions were carried aut at 70° C. Yields were calculated after drying the fish solids for 16 hr at 70° C at 25 inches of vacuum.

RESULTS AND DISCUSSION

The effect of autolytic activity on the subsequent yield of FPC is shown in Figure 6. It can be seen that while the acidified samples showed the greatest loss of yield with time of autolysis, the largest yield losses occurred during the first 20 min. The pH values, time, and temperature were arbitrarily chosen to simply demonstrate the effect of autolysis with respect to yield. They do, however, show a close correlation with the autolysis data shown in the previous experiment. For example, in referring to Figures 1 through 5, it can be seen that the rate of NPN formation is greatest during the first 20 min regardless of pH and temperature. Several investigators (Whaley, 1966; Sen, Sayanarayana Rao, Kadkol, Krishnaswamy, Venkata Rao, and Lahiry, 1969) have proposed acidifying comminuted fish prior to processing without taking into account the effect of protein losses due to autolysis. The combined experiments presented here clearly demonstrate the need to control temperature and pH, both before and during processing.

SUMMARY AND CONCLUSIONS

The above study shows that the endogenous proteolytic enzymes in fish hydrolyze the proteins into subunits that are not coagulable by

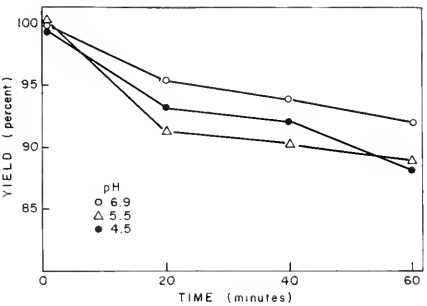

FIGURE 6.—Effect of protein autolysis on the yield of PFC made from inside hake. Autolysis was allowed to proceed for 0, 20, 40, and 60 min at 50° C prior to preparation of FPC.

the isopropanol concentration normally used in the preparation of FPC. The preparation of FPC made from autolyzing hake showed that the yield bore a close inverse relation to the degree of autolysis.

The autolysis rates in Pacific herring and hake were related to pH and temperature, the rates showing an increase with increasing temperature and a decreasing pH. Maximum activity was reached at about 50° C. Inactivation of the enzymic systems occurred when temperatures exceeded 70° C.

Since the economic success of any method that is used for the preparation of FPC is largely dependent on the yield of finished product obtained from a given amount of raw material, autolysis rates are a process parameter that should be closely controlled.

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EQUIPMENT FOR HOLDING AND RELEASING PENAEID SHRIMP DURING MARKING EXPERIMENTS[1]

Personnel of the National Marine Fisheries Service Biological Laboratory at Galveston, Texas, have conducted numerous mark-recapture experiments to obtain information on the movement, growth, and mortality of penaeid shrimp. These experiments were carried out under a variety of conditions at sea and in coastal bays. Several types of specialized equipment were developed to overcome problems of holding, handling, and releasing shrimp during the marking phase of these experiments. Some of this equipment has been described previously by Costello (1964). Holding tanks, a cooling unit, and two devices used to transport shrimp to the sea floor are described here.

Holding Facilities

A number of factors were considered in the design of tanks for holding shrimp. Construction materials had to be relatively light in weight, require little maintenance, and be nontoxic to shrimp. Provisions also were needed to permit rapid water exchange, minimize water turbulence within tanks, and control water temperature. The tank design in Figure 1 meets these needs and has proved successful for both sea- and land-based operations. It is constructed of light gray fiberglass with wood reinforcement and weighs about 114 kg (250 lb.). Advantages of the light color are that it reflects heat and makes shrimp easily visible in the tank. To permit rapid drainage or water exchange, a polyvinyl chloride (PVC) pipe, 7.6 cm (3 inches) in diameter, is molded into each end of the tank near the bottom. Filter screens, used to prevent loss of shrimp in outflowing water, have a large surface area to minimize clogging. A one-quarter section of PVC pipe, 7.6 cm (3 inches) in diameter, is molded to the top of the tank at each end as a splash rail to reduce spillage.

Five sets of guides in the tank support baffles that reduce water turbulence at sea and are used to separate groups of shrimp in a tank (Fig. 1). The baffles have a frame of aluminum flashing covered with sheets of patterned aluminum 0.063 cm (0.025 inch) thick.

During field use, a series of two to four tanks are linked to provide either recirculating water or continually flowing new water. The pump used depends on the volume of water required. Normally, we use a cast-iron pump powered by a 0.5-hp electric motor (110-220 v) that discharged 114 to 132 liters (30 to 35 gal) per min. As the water is discharged into the tanks, it passes through siphon filler-drain nozzles (Costello, 1964) which draw air into the circulation system and aerate the water. The aeration unit (Fig. 2), made of 1.9-cm (0.75-inch) pipe, may be attached temporarily at any convenient place on the tank. The amount of air that enters the water is regulated by valves in each air line.

Because it is difficult to keep shrimp alive when water temperatures exceed about 27° C (80° F), cooling units are used to lower and maintain temperatures in holding tanks. A cooling unit of our own design is shown in Figures 3 and 4. The casing consists of a PVC pipe, 25.4 cm (10 inches) inside diameter, 45.7 cm (18 inches) long, and 0.9 cm (0.37 inch), thick, and top and bottom pieces of PVC flat stock, 30.5 by 30.5 by 1.3 cm (12 by 12 inches by 0.5 inch) with circular grooves 0.6 cm (0.25 inch) deep. O-ring gaskets that fit the grooves prevent leakage of water. The refrigerant coil is made from 0.9-cm (0.37-inch) diameter stainless steel tubing, 9 m (30 ft) long. A thermostat sensor receptacle, inserted through the top

[1] Contribution No. 304, National Marine Fisheries Service Biological Laboratory, Galveston, Texas.

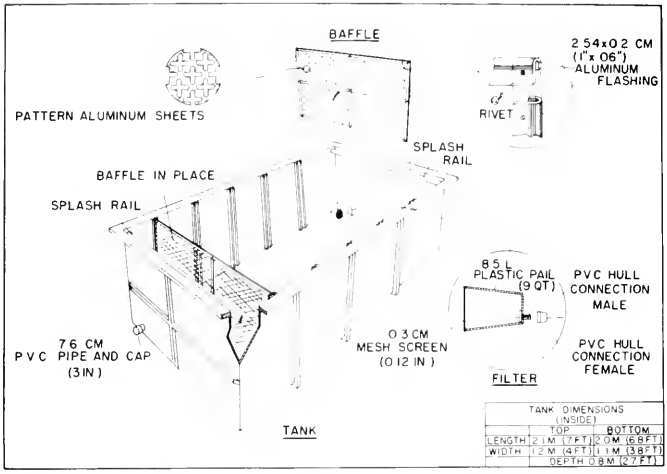

FIGURE 1.—The holding tank, baffles, and filters used in shrimp marking experiments.

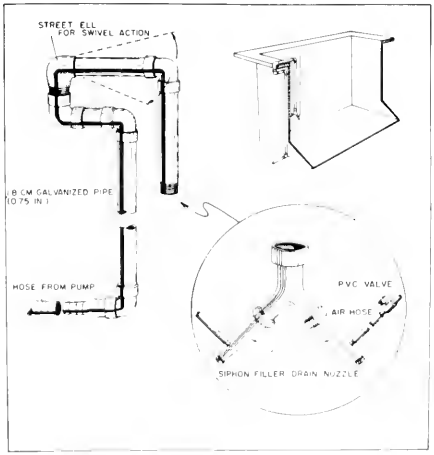

FIGURE 2.—Aeration unit and siphon filler-drain nozzle through which water enters tank.

plate of the cooling tank, consists of a piece of 0.6-cm (0.3-inch) diameter stainless steel tubing, 20.3 cm (8 inches) long, and is sealed at one end. The top of the chilling tank is reinforced by a 30.5 by 30.5 cm (12 by 12 inches) frame made from angle aluminum stock 3.8 by 3.8 by 0.6 cm (1.5 by 1.5 inches by 0.25 inch).

Two experiments were completed to determine the cooling capability of the unit. Water was recirculated through the chilling unit at rates of 114 to 132 liters (30 to 35 gal) per min, and thermographs recorded water and air temperatures (Fig. 5). The temperature attained after 24 hr was about 15.6° C (60° F) and was lower than that required for shrimp-marking procedures. Field observations have indicated that water temperatures can be maintained within 2° C (4° F) of desired levels, irrespective of fluctuations in air temperatures.

A table top with plastic pans 33.0 cm (13 inches) long, 38.1 cm (15 inches) wide, and 13.97 cm (5.5 inches) deep equipped for continuous water circulation (Fig. 6) slides over the lip of the holding tank and extends about 5 cm (2 inches) beyond the ends of the tank. When in

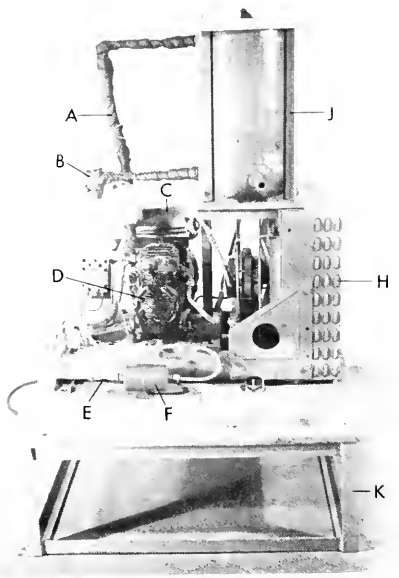

FIGURE 3.—One-hp single-phase compressor and condensing unit (12,000 BTU factory rated) attached to a PVC chilling tank and mounted on angle iron stand. A. vibration joint; B. expansion valve; C. thermostat control; D. compressor; E. sight glass; F. dryer; H. condensing unit; J. chilling tank; K. angle iron stand.

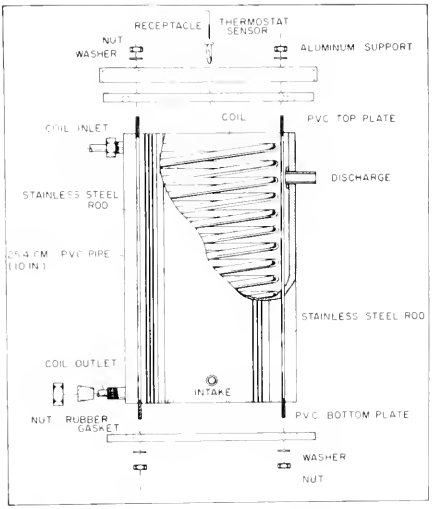

FIGURE 4.—Details of the chilling tank assembly.

place, the table top serves as a work area for staining and tagging shrimp which are held in the pans.

Equipment for Releasing Shrimp

Three types of release devices have been developed to protect shrimp from exposure to predation during their return to the sea floor. Costello (1964) described a release box that is lowered to the bottom with a winch and opened by

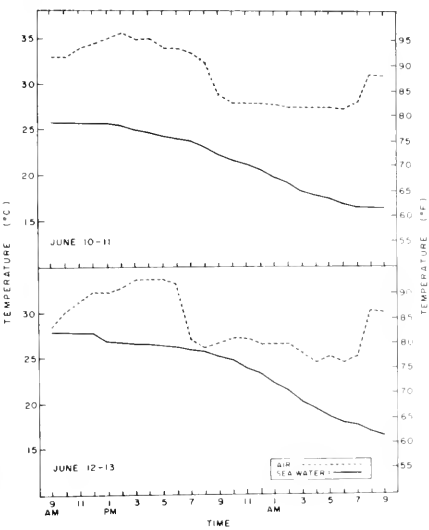

FIGURE 5.—Reduction of water temperatures in a 1,892-liter (500-gal) tank compared to surrounding air temperatures during trials with the chilling unit.

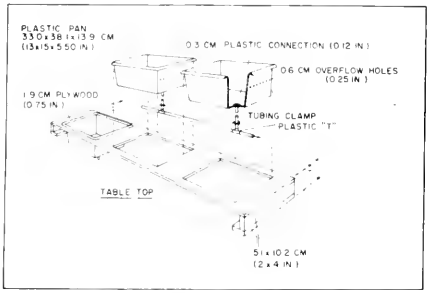

FIGURE 6.—Removable table top and holding pans equipped for continuous water circulation.

a messenger dropped down the cable. Use of this device is restricted to large vessels equipped with a winch, and requires that the vessel be stopped when shrimp are released. To circumvent these requirements, we designed an expendable release canister that can be put overboard while a vessel is underway and a release tube for use in shallow water.

The canister (Fig. 7) is constructed of high-impact styrene plastic formed into a hollow cylinder. Tabs on each of the styrene plastic end pieces have holes to accommodate retaining rods used in assembling the canister. Assembly and loading are accomplished in a cradle attached to the inner wall of a holding tank so that shrimp will remain submerged until the canister is ready to be put overboard. Slots in the canister allow it to fill with water.

A salt block, a rubber band, and a paper clip constitute the release mechanism. This mechanism is set by folding together the two ends of the styrene plastic sheet (thus forming a cylinder) and securing them with a rubber band. When ends A and B (see canister, Fig. 7) are folded, the paper clip is inside the canister with the attached rubber band inserted through a hole (end A) and a slot (end B). The salt block is then inserted in the loop formed by the rubber band, and the retaining rods are removed.

A cement weight (1.1 kg or 2.5 lb.) is attached and the canister is lowered to the water surface and released. When the salt block dissolves, tension in the canister wall pulls the rubber band from the slot (end B) and the canister disassembles, releasing the shrimp. Although never observed during the actual release of shrimp in offshore waters, this release devise was tested in shallow estuarine waters and in the laboratory. In all tests it performed as expected. The canister accommodates up to 100 shrimp that

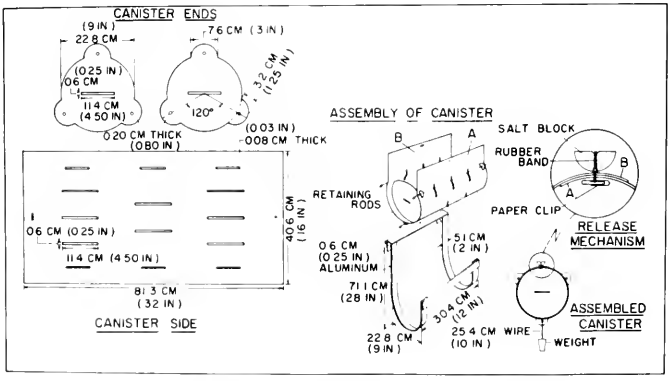

FIGURE 7.—Disposable release canister showing release mechanism, loading cradle, and method of assembly.

are released on the sea bottom within 5 to 15 min from the time the unit enters the water, depending on the size of the salt block.

The release tube (Fig. 8), intended for use in shallow water, consists of two telescoping aluminum pipes, each about 3 m (10 ft) long. To release shrimp, the outer pipe is lowered to the bottom and shrimp are poured from a pail into the funnel. After each pail of shrimp is poured into the unit, the apparatus is flushed with several pails of water to insure that shrimp do not remain in the tube. The pouring and flushing of one pail of shrimp usually take about 1 min.

The new equipment described herein and the improved techniques for staining and tagging described by Neal (1969) enabled us to hold, mark, and release large numbers of shrimp. We can now process between 1,500 and 3,000 shrimp per day, depending on the type of mark used.

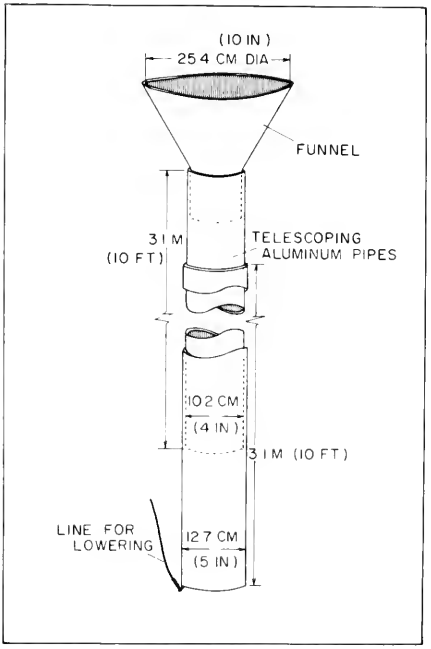

FIGURE 8.—Release tube used to place marked shrimp on the bottom.

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AN ADULT BLUEFIN TUNA, *Thunnus thynnus*, FROM A FLORIDA WEST COAST URBAN WATERWAY[1]

The bluefin tuna, *Thunnus thynnus* (Linnaeus), is a wide-ranging pelagic species occurring in most tropical and temperate seas (Gibbs and Collette, 1966: 119). In the Gulf of Mexico exploratory and commercial catches have been limited to the northern, western, and central parts, from waters beyond the continental shelf. The collection of a large adult from the Florida west coast represents a new record for the Florida shelf.

The specimen, a female, was captured by local fishermen with harpoons in a waterway at Hudson, Fla., (lat 28°21′24″ N, long 82°42′42″ W) on 10 May 1970. It weighed 239 kg (525 lb.), was 244 cm (96 inches) in fork length and 168 cm (66 inches) in girth, and appeared to be in healthy but lean condition, characteristic of post-spawning fish in May on the Bahama Banks (Rivas, 1955: 139).

Histological examination of gonadal tissue sectioned at 6 μ and stained with Harris hematoxylin and Eosin Y showed early and late atretic

[1] Contribution No. 154.

body formations, indicating recent spawning. The stomach contained a mussel, *Brachidontis recurvus* (Rafinesque), a piece of marl (no doubt ingested accidentally), and a digenetic trematode; gills and other tissues were free of parasites.

The fish had entered the waterway through a shallow navigation channel from the adjacent grass flats. The waterway, a network of channels cut through marl, consists of several 161 m by 15 m "fingers" branching from a 1370 m by 22 m central channel, with depths averaging less than 5 m at low tide. Surface salinity in the waterway, tested at a subsequent low tide, was 27.0 ‰; surface temperature in the adjacent Gulf was 26° C.

This occurrence, although admittedly irregular, may help to fill a gap in our emerging picture of the origin and distribution of Gulf of Mexico bluefin tuna stocks.

Bluefin tuna are taken from the Greater Antilles in spring, with substantial numbers of large adults being reported from the Windward Passage in April (Bullis, 1955: 6). During May they begin their dramatic migration through the Straits of Florida toward the summer feeding grounds (Rivas, 1954, 1955).

The occurrence of large bluefins at Grand Cayman and east of Cozumel in April (Bullis and Mather, 1956: 9) suggests that at least a component of these Caribbean stocks may undertake a similar northward movement through the Yucatan Straits and into the Gulf of Mexico. The occurrence of ripe or nearly ripe females in the Gulf in May and of small juveniles (less than 8 cm) in the northern Gulf in late May and early June (Mather, 1962: 5) implies that these stocks spawn in the Yucatan Straits or in the Gulf of Mexico proper. Our spent female on the Florida shelf could be from the Caribbean stock or from a stock wintering in the Gulf (Bullis, 1955: 13; Wathne, 1959: 16).

We are indebted to Gordon D. Marston, *St. Petersburg Times*, for informing us of the incident, to Bud and Marvin Mattix and others for allowing us to examine the fish and viscera, to Richard B. Roe for providing information on specimens collected during exploratory fishing by U.S. Fish and Wildlife Service vessels in the Gulf of Mexico and Caribbean, to Alice Gennette for preparing histological sections, and to Frank J. Mather, III for critically reviewing the manuscript.

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SWIMMING SPEED, TAIL BEAT FREQUENCY, TAIL BEAT AMPLITUDE, AND SIZE IN JACK MACKEREL, *Trachurus symmetricus*, AND OTHER FISHES

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ABSTRACT

The tail beat frequency and tail beat amplitude of jack mackerel, *Trachurus symmetricus*, 4.5 to 27.7 cm were measured at speeds of 15 to 212 cm/sec. Tail beat amplitude was a constant proportion of length at all speeds but tail beat frequency changed with speed; thus speed depended only on frequency of the tail beat and length. A simple mathematical model for estimating swimming speed from tail beat frequency and fish length was derived from the *Trachurus* data and applied to data for three marine fish — *Scomber japonicus*, *Triakis henlei*, and *Sardinops sagax* — and to data for freshwater fish from the literature. The general form of the model was $V - V_0 = L(KF - F_0)$ where V is fish speed, V_0 is length-dependent minimum swimming speed at minimum tail beat frequency F_0, and L is fish total length. The model represented a major improvement over previous equations because it provided an unbiased correction for length, was sensitive to specific differences, and provided a more accurate estimation of speed.

Of the variables that determine the swimming speed of a fish, the size of the fish, the frequency of the tail beat, and the amplitude of the tail beat are among the most important. Knowledge of the relationships between swimming speed and these variables is important not only for an understanding of the mechanism of locomotion in fish but because it may be used to forecast maximum swimming speeds (Bainbridge, 1958), to estimate swimming speeds indirectly by analysis of tail beat frequencies, and possibly to estimate fish size and make specific identifications of fish targets with doppler Continuous Transmission Frequency Modulated sonar (Hester, 1967).

Bainbridge (1958) described the relationship between tail beat frequency, tail beat amplitude, and size for three species of freshwater fish: dace, *Leuciscus leuciscus*; trout, *Salmo gairdneri* (*S. irideus*); and goldfish, *Carassius auratus*. He concluded that the amplitude of the tail beat increased with the tail beat frequency to about 5 tail beats/sec and thereafter became constant. Speeds above 5 beats/sec were dependent only on the frequency of the tail beat and the length of the fish. The relationship between speed, frequency, and length above 5 beats/sec was nearly the same in the three species studied; consequently, he used a single equation to express this relationship for all three species. No similar study exists for marine fish although some measurements of tail beat frequency and amplitude have been made incidental to other studies. Yuen (1966) measured the tail beat frequency of skipjack tuna, *Katsuwonus pelamis*, and yellowfin tuna, *Thunnus albacares*, from cine photographs taken from the viewing port of a research vessel and Magnuson and Prescott (1966) measured the tail beat frequency of Pacific bonito, *Sarda chiliensis*, from cine photographs taken through a window in an oceanarium. The slopes of the lines relating tail beat frequency to speed in body lengths per second for skipjack and yellowfin tunas and bonito were sufficiently different from those of Bainbridge (1958), for Hester (1967) to speculate that species might be identifiable by this relationship. The measurements were taken from lateral photographs of free-swimming schools; thus the tail beat amplitude and the absolute

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size of the fish were not measured and tail beat frequency was measured over a limited speed range. Fierstine and Walters (1968) measured both tail beat frequency and amplitude of wavyback skipjack, *Euthynnus affinis*, from dorsal cine photographs of free-swimming fish in circular swimming pools but only five, one-beat sequences of swimming were analyzed.

The objective of the present study was to determine the relationships between swimming speed, fish length, tail beat amplitude, and tail beat frequency in a pelagic marine fish, jack mackerel, *Trachurus symmetricus*. To accomplish this objective, dorsal cine photographs were taken of fish swimming in currents of different speeds in a specially designed activity chamber. For comparative purposes tests were also run on three other marine fish: chub mackerel, *Scomber japonicus;* Pacific sardine, *Sardinops sagax*; and a shark, *Triakis henlei*.

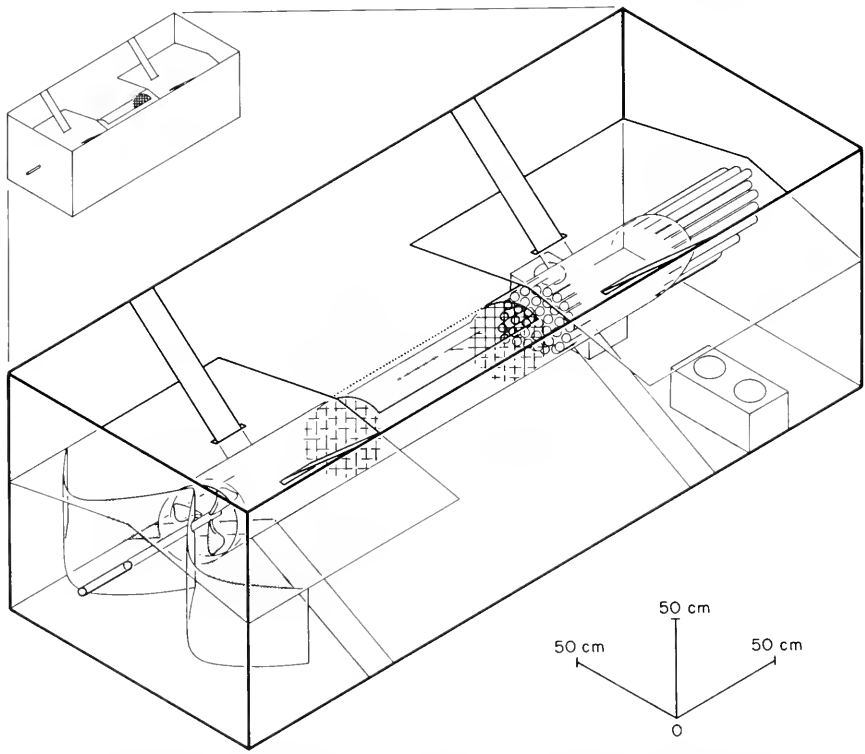

FIGURE 1.—Apparatus used to measure swimming speeds of fishes. Inset upper left apparatus shown with opaque walls; center, isometric, three dimensional scale drawing, walls, deflectors and other structures are shown as transparent for purposes of illustration; arrows indicate direction of current flow; and lower right, scale for vertical and horizontal planes of drawing.

APPARATUS

Swimming speeds were measured in an activity chamber (Figure 1) built after that of Beamish (1966). A fiber glass tube 230 cm long and 41 cm in diameter was immersed in an open bath. An 80-cm section of tube was the swimming compartment. The compartment had metal screens at the ends and a transparent acrylic plastic hatch which conformed to the contours of the tube. The walls of the tube within the swimming compartment were white and had black stripes spaced at 5.0-cm intervals to provide a visual reference for the swimming fish. Water velocity in the chamber was regulated by the speed of a 39-cm propeller driven by a variable speed 50-hp motor and by changing the screens at the two ends of the swimming compartment. Water was drawn into the compartment from the bath over deflectors, and through baffles and screens. The screens, baffles, and deflectors reduced turbulence and provided water of relatively uniform velocity throughout the swimming compartment. Their arrangement and design were determined empirically by measurement of the horizontal and vertical distribution of flow in the chamber.

The speed range of the apparatus was 12 to 212 cm/sec. The full range was obtained by changing the screens at the ends of the swimming compartment. A velocity range of 12 to 69 cm/sec was obtained when screens of 39% open area were used, one of 15 to 139 cm/sec for screens of 56% open area, and one of 19 to 212 cm/sec for screens of 75% open area.

A digital voltmeter measured to the nearest millivolt the voltage produced by a voltage generator attached to the propeller shaft. The voltage produced by the generator was proportional to propeller revolutions and was used to regulate them. An impeller flowmeter (Marine Advisers Inc., Model B-7C)[2] was used to relate propeller revolutions in volts to water flow in the apparatus. The meter sampled an area 7.6 cm in diameter and had an accuracy of ± 2.5 cm/sec when moved through static water at a known speed (for a description of the meter and a calibration curve see Olson, 1967).

Propeller revolution was related to water speed in the chamber by three series of calibrations, one for each of three screen types used. Nine to 19 different speed levels were measured in each series of calibrations. More levels were required for slow speed ranges than for fast ones because the response of the flowmeter was nonlinear at low speeds. At each level water speed was measured at 12 different radial positions midway in the swimming compartment. The speed of the water at a given level was the average of the 12 measurements, adjusted for the extent of the area sampled by the meter (Tranter and Smith, 1968). Variation among the 12 sampling points did not exceed ±10% of the mean speed and was usually much lower. The relationship between mean water speed in the chamber and propeller revolutions was linear, and the error in estimating the mean water speed from revolutions did not exceed ± 0.2 cm/sec. Thus, the principal sources of error in estimating the speed of the water in which a fish swam were the possible 10% variability in flow within the chamber and the ± 2.5 cm/sec accuracy of the flowmeter.

Fish swimming in the compartment were assumed to be swimming at the mean speed of the water in the compartment. They were photographed from above with a 16 mm high-speed motion picture camera at speeds of 64 to 200 fps. Camera speed was adjusted to provide about 10 frames per complete tail beat. A viewing box floated on the water surface above the swimming compartment to eliminate distortion in the photographs caused by ripples.

METHODS

Film was analyzed by use of a coordinate reader and digitizer (Hunter, 1966) and a computer program was used to calculate tail beat frequency and tail beat amplitude from the digitized information. One tail beat was one complete oscillation of the tail, and the tail beat frequency was the number of beats per second. Tail beat amplitude was the distance in centimeters between the lateral most excursion of

[2] Reference to commercial products does not imply endorsement.

the tip of the tail measured perpendicular to the axis of progression plus the mirror image of that measurement on the other side of the axis of progression.

The film sequences selected for analysis were usually ones in which the fish held a constant position in the current and swam steadily. Occasionally at higher speeds it was not possible to obtain such a sequence because the fish did not maintain a constant position but rather accelerated and decelerated. In this case a sequence was chosen in which no net movement existed between the beginning and end of the sequence although the fish moved slightly forward and backward within the sequence. Usually 5 complete tail beats were analyzed per speed level but occasionally as few as 2.5 and as many as 11 were analyzed.

Sixteen speed levels were used in the experiments; seven of the levels, 15 to 60 cm/sec, were graduated at intervals of 25% of the preceding level and nine of the levels, 69 to 212 cm/sec were graduated at intervals of 15%. A speed level interval greater than 10% was used because of the possible 10% variability in flow within the swimming compartment.

A grand total of 176 speed tests was analyzed for 14 jack mackerel, varying in total length from 4.5 to 27.7 cm. Owing to the differences in length, no fish was able to swim at all levels. All 14 fish swam at five levels, 24, 30, 38, 48, and 60 cm/sec, and all but the two smallest fish swam at the next five higher levels, 79, 91, 105, 121, and 139 cm/sec. Only fish of 16 cm or larger were tested at speeds above 139 cm sec and only those less than 16 cm were tested at 15 cm/sec.

Other species were tested for comparative purposes but fewer observations were made. Seventy-four swimming sequences of five *Scomber*, 26.3 to 32.2 cm total length, were analyzed, nine sequences of five *Sardinops*, average length 13.6 cm, and seven sequences of one *Triakis*, 23.6 cm.

All fish were tested singly except for *Sardinops*, which was tested in a group of five. Fish were held in the swimming compartment at a low speed for about 30 min before an experiment began. Seawater temperature ranged from 17.0° to 19.5° C among experiments but did not vary over a degree within an experiment.

RESULTS

The tail beat amplitude of *Trachurus* did not change with speed but was constant at all speed levels and was directly related to length (Figures 2 and 3). Tail beat frequency, on the other hand, changed with speed and, therefore, was the only speed modulator measured in these experiments. In all species studied the relationship between tail beat frequency and velocity

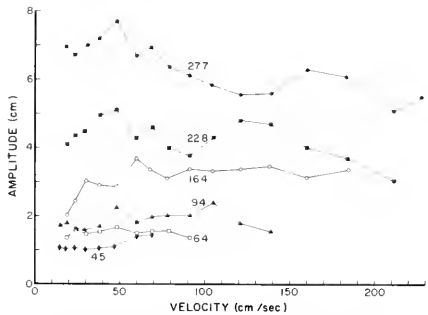

FIGURE 2.—Tail beat amplitude at various speeds for six *Trachurus*, 4.5 to 27.7 cm total length.

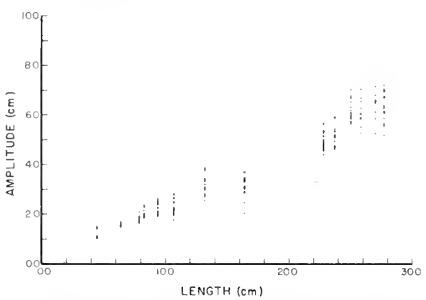

FIGURE 3.—Relationship between tail beat amplitude and total length for 14 *Trachurus*, 4.5 to 27.7 cm total length. ($A = 0.23177L$, $s_{yx} = 5.068$, and $N = 176$.)

was linear throughout the range of test speeds, but the slope and intercept of the regression lines varied with fish length (Figure 4, Table 1).

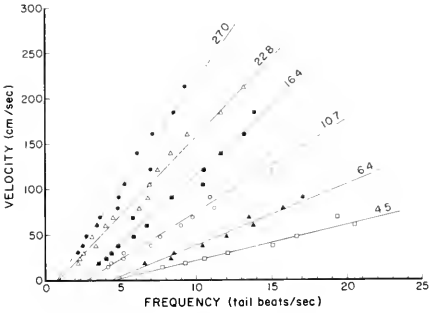

FIGURE 4.—Relationship between speed and tail beat frequency for six *Trachurus*, 4.5 to 27.0 cm total length. Equations for regression lines shown in figure are given in Table 1.

TABLE 1.—Standard deviation (s_{yx}), intercept (a), and slope (b) for regression of speed (cm/sec) on tail beat frequency and slope and intercept for regression of speed/length on frequency for *Trachurus*.

Length (cm)	N	Speed (cm/sec) on frequency			Speed/length on frequency	
		a	b	s_{yx}	a	b
4.5	8	−17.786	3.996	3.889	−3.952	0.988
6.4	9	−29.761	6.838	4.284	−4.650	1.068
7.9	11	−37.527	8.326	4.135	−4.624	1.054
8.3	11	−39.736	9.374	10.096	−4.787	1.129
9.4	13	−35.241	8.953	5.258	−3.749	0.952
10.7	13	−29.012	10.431	5.612	−2.781	0.975
13.2	13	−19.860	11.514	10.762	−1.504	0.873
16.4	14	−32.541	14.780	9.060	−1.984	0.901
22.8	15	−15.407	17.497	6.945	−0.676	0.767
23.7	13	−13.723	19.753	6.457	−0.579	0.833
25.0	15	−33.403	23.394	11.832	−1.336	0.936
25.8	13	−23.220	21.730	8.734	−0.900	0.842
27.0	13	−20.521	23.960	9.157	−0.760	0.887
27.7	15	−11.240	20.509	9.182	−0.406	0.740
Total	176					

The length-dependent differences in intercept were probably a function of differences in minimum speed and minimum tail beat frequency. Fish have a minimum tail beat frequency and a minimum swimming speed below which they cannot swim by movement of the caudal fin and these minima were a function of body length. In the past, speed was scaled directly to length; that is, speed was divided by length and regressed on frequency (Bainbridge, 1958; Magnuson and Prescott, 1966; Yuen, 1966). Our data suggest, however, that division of speed by length would introduce bias because of the existence of a minimum speed and tail beat frequency different from zero, the dependence of minimum speed on length, and possible length-dependent differences in the slope of the regression of speed on frequency. For example, when we divided speed by length, size-dependent differences in intercept and possibly the slope still existed (Table 1, last two columns). In addition a curvilinear trend is introduced at low speeds in the combined data because of the lack of an intercept (minimum speed) correction. Thus an equation that relates speed to tail beat frequency for fish of different length must include an adjustment for size-dependent differences in minimum swimming speed, minimum tail beat frequency, and perhaps also for size-dependent differences in the slope coefficient.

The existence of size-dependent variables introduces certain problems in the interpretation of these data because of the possibility that specific differences in size dependency may exist. For example, differences exist among species in the coefficients used to relate size to various swimming characteristics such as burst and sustained speeds (Bainbridge, 1960) but it is uncertain whether or not these differences reflect real specific differences or if they are simply differing estimates of a common coefficient. Owing to the great variability inherent in swimming speed studies and because of the sensitivity of the length coefficients to the size range of animals in the sample, these two alternatives are equally plausible. In addition, specific differences in the relationship between size and swimming functions may also depend on the particular function considered (Bainbridge, 1960). For example, the coefficient relating size to maximum sustained speed may be different from the one that relates size to beat frequency or burst speed. Thus, species may differ from one another in the way each swimming function is related to size. If such specific differences exist

then direct comparisons between species are impossible, but if they do not exist then a general model can be derived from our data which can be used to make specific comparisons. In the interpretation of our data on *Trachurus* we will consider the alternatives, Case I where all swimming functions are related to size on a species-specific basis, and Case II where swimming functions are proportional to the same power of length in different species.

To evaluate Case I where length coefficients are considered to be species-specific we regressed speed on frequency by the general relationship,

$$V = \alpha_1 L^{\alpha_2} + \alpha_3 L^{\alpha_4} * F$$

where V is swimming speed in centimeters per second, F is tail beat frequency in beats per second, L is total length in centimeters and $\alpha_1 L^{\alpha_2}$ is the intercept function and $\alpha_3 L^{\alpha_4}$ is the slope function for the tail beat frequency-swimming speed relationship. Estimates were obtained by use of Marquardt's Algorithm for fitting nonlinear models (Conway, Glass, and Wilcox, 1970). For *Trachurus* the 90% support-plane confidence intervals (Conway et al., 1970) for α_3 and α_4 were $0.72 < \alpha_3 < 1.82$ and $0.72 < \alpha_4 < 1.01$ where $\hat{\alpha}_3 = 1.28$ and $\hat{\alpha}_4 = 0.86$.

To evaluate Case II where length coefficients are the same for all fish we assumed the slope coefficient α_4 equaled one. When $\alpha_4 = 1$, $\hat{\alpha}_3 = 0.86$ with 90% confidence limits of $0.79 < \alpha_3 < 0.91$. On the basis of the *Trachurus* data alone there seems to be little or no difference between the use of unity for the slope coefficient or use of the estimated value of 0.86. The similarity in the two estimates is apparent when the actual fish lengths are substituted into the two equations and the two sets of slopes are compared (Table 2, columns 1 and 2).

We fitted the Case I model to four additional species to determine the extent they differed in the length coefficient for the slope in the speed-tail beat frequency relationship. Used in this comparison were data we collected on *Scomber*, and data presented in scatter plots of velocity and frequency for individual *Carassius*, *Salmo*, and *Leuciscus* by Bainbridge (1958). We used the *XY* digitizer to transcribe Bainbridge's data onto cards. We may have failed to interpret correctly some of the overlapped points in his graphs but the effect of these errors on the statistical parameters we estimated would be negligible. We did not use the data presented by Magnuson and Prescott (1966), Yuen (1966), or Fierstine and Walters (1968) because in these studies the absolute speeds and the lengths of the fish were unknown.

Our estimates of the slope coefficient for the speed-tail beat relationship, $\hat{\alpha}_4$, varied from 0.76 in *Salmo* to 1.22 in *Carassius*, and the 90%

TABLE 2.—Slopes for the speed-frequency relationship for individual *Trachurus* when the general relationship is slope $= 1.28\ L^{0.86}$ (Case I) and when slope $= 0.86L$ (Case II); estimated minimum speed (V_0) for each fish when $V_0 = 0.80L^{2/3}$; lowest observed test speed (V_{obs}); the minimum tail beat frequency (F_0) estimated by substitution of V_0 the Case II equation for each fish; and the lowest observed tail beat frequency (F_{obs}).

Length (cm)	$b = 1.28L^{0.86}$ Case I	$b = 0.86L$ Case II	$V_0 = 0.80L^{2/3}$	V_{obs}	$F_0 = 3.98L^{-1/3}$ Case II	F_{obs}
4.5	4.67	3.87	2.19	14.9	2.41	7.77
6.4	6.32	5.50	2.77	18.9	2.14	6.60
7.9	7.57	6.79	3.19	24.0	2.00	6.75
8.3	7.90	7.14	3.30	24.0	1.97	6.68
9.4	8.79	8.08	3.59	15.1	1.88	5.22
10.7	9.83	9.20	3.92	15.0	1.81	4.18
13.2	10.98	11.35	4.51	15.0	1.68	2.91
16.4	14.19	14.10	5.21	19.0	1.56	3.56
22.8	18.84	19.61	6.50	18.9	1.40	2.18
23.7	19.48	20.38	6.67	24.1	1.38	1.86
25.0	20.39	21.50	6.91	19.8	1.36	2.46
25.8	20.95	22.19	7.06	24.7	1.35	2.11
27.0	21.78	23.22	7.28	31.4	1.33	2.16
27.7	22.27	23.82	7.40	19.0	1.32	1.78

support-plane intervals for $\hat{\alpha}_4$ in all species included unity (Table 3). Clearly if a common slope-length coefficient exists among these species, it must be close to 1. We conclude the assumption of unity for the slope-length coefficient is an acceptable practice and that it appears to introduce no significant bias in the species studied.

We now turn to the problem of estimation of the length-dependent coefficient for the intercept of the speed-tail beat relationship, that is, α_2. We noted previously that the biological significance of the existence of an intercept different from zero in the speed-frequency relationship may be that fish have a minimum speed below which they cannot swim by movements of the caudal fin. If we assume that the intercept is a function of the minimum swimming speed of a fish we can make an independent estimate of the intercept coefficient using an equation derived by Magnuson (1970) for estimation of the minimum swimming speed (V_0) of *E. affinis*. A somewhat simplified form of his equation is:

$$V_0 = \left(1 - \frac{D_e}{D_f} \right)^{1/2} * \left[\frac{g * Ma}{(C_{lf} A_{ft}) * \frac{\rho}{2}} \right]^{1/2}$$

where D_e is 1.025 (the density of sea water), D_f is the density of the fish, g is 980 cm/sec (the acceleration of gravity), Ma is mass of fish in air, C_{lf} is the coefficient of lift for the pectorals (assumed to equal 1), A_{ft} is the total lifting area of the extended pectoral fins in square centimeters, and ρ is the density of sea water, 1.025 g/cc. If we let $Ma = 0.004407 L^{3.21215}$ (the length-weight relationship for *T. symmetricus*, $N = 264$, unpublished data, Na-

TABLE 3.—Estimates of length coefficients for slope and intercept for five species of fish.

Species	$V = \alpha_1 L^{\alpha_3} + \alpha_3 L^{\alpha_4} * F$	90% support-plane interval[1]
Trachurus symmetricus	$V = -44.57L^{-0.24} + 1.28L^{0.56} * F$	$-111.18 < \alpha_1 < 22.65$ $-0.80 < \alpha_2 < 0.31$ $0.73 < \alpha_3 < 1.82$ $0.72 < \alpha_4 < 1.01$ $s_{vf} = 11.41$
Scomber japonicus	$V = -0.56L^{0.55} + 1.20L^{0.83} * F$	$-160.78 < \alpha_1 < 159.66$ $-84.14 < \alpha_2 < 85.25$ $-8.15 < \alpha_3 < 10.57$ $-1.46 < \alpha_4 < 3.13$ $s_{vf} = 21.42$
Leuciscus leuciscus[2]	$V = -3.36L^{0.40} + 0.80L^{0.98} * F$	$-14.95 < \alpha_1 < 8.24$ $-0.84 < \alpha_2 < 1.65$ $0.47 < \alpha_3 < 1.12$ $0.83 < \alpha_4 < 1.12$ $s_{vf} = 7.81$
Salmo gairdneri[2]	$V = -1.77L^{0.64} + 1.40L^{0.76} * F$	$-14.79 < \alpha_1 < 11.26$ $-0.70 < \alpha_2 < 2.97$ $0.33 < \alpha_3 < 2.46$ $0.52 < \alpha_4 < 1.00$ $s_{vf} = 14.16$
Carassius auratus[2]	$V = -0.12L^{1.52} + 0.40L^{1.22} * F$	$-0.89 < \alpha_1 < 0.64$ $-0.50 < \alpha_2 < 3.53$ $0.17 < \alpha_3 < 0.63$ $1.01 < \alpha_4 < 1.44$ $s_{vf} = 5.54$

[1] Simultaneous confidence intervals for all parameters (Conway, Glass, and Wilcox, 1970).
[2] Data from Bainbridge (1958).

tional Marine Fisheries Service, La Jolla, Calif.). Area $= 0.02811L^{1.9124}$ (lifting area of pectorals of *T. symmetricus* was equal to twice the pectoral fin area), and $D_f = 1.03$ (the density of *T. trachurus* from Alexander (1959b), a closely related species to *T. symmetricus*), we obtain an estimate of $V_0 = 0.86L^{0.65}$ for *Trachurus*. This value is considerably below $10L^{0.50}$ obtained for *Euthynnus* by Magnuson (1970). The minimum swimming speed of *Trachurus* would be expected to be lower than that of *Euthynnus* because *Euthynnus* lacks a swim bladder and has a high specific gravity. Indeed, the minimum speed of *Euthynnus* is close to the endurance speed of many fishes with swim bladders (Magnuson, 1970).

In all other species except *Carassius* estimation of minimum speed was not possible because we had few or no estimates of the variables required in Magnuson's equation. In *Carassius* we used the specific gravity for carp, 1.002 g/cc (Alexander, 1959a), the pectoral fin area relationship of Area $= 0.02811L^{1.9124}$ from data we collected on seven *Carassius* 4.6 to 22.5 cm total length (the lifting area of the pectorals equaled twice the pectoral fin area) and the length-weight relationship of $Ma = 0.0065L^{3.20}$ for the seven *Carassius*. The estimate of minimum swimming speed V_0 for *Carassius* from these data was $0.87L^{0.65}$. This estimate was nearly the same as the one estimated above for *Trachurus* and it had the same coefficient of length. Thus, in *Trachurus* and in *Carassius* the minimum speed coefficient of length or, in our equation, the intercept coefficient $\hat{a}_2$, was 0.65.

That the length coefficient for the intercept was the same in *Carassius* and *Trachurus* supports the basic assumption of the Case II model, that is, the existence of common length coefficient among different species. To further test this assumption we estimated the intercept coefficient by fitting the combined data from all five species listed in Table 3 to the reduced Case II model

$$V = \alpha_1 L^{\alpha_2} + \alpha_3 L * F$$

The estimate α_2 for the combined data was 0.68 with 90% confidence limits of $-0.56 < \alpha_2 < 1.92$. Although the limits were wide, the estimate was very close to the other independent estimate for *Carassius* and *Trachurus* and suggests that the true value may be close to 2/3.

The evidence we presented supported the use of the Case II equation and the use of 2/3 for the length-dependent coefficient for the intercept function and of unity for the length-dependent coefficient of the slope function. Thus, we fit the reduced Case II model

$$V = \alpha_1 L^{2/3} + \alpha_2 L * F$$

to the data from each of the five species listed in Table 3 and to that from two additional species *Triakis* and *Sardinops* for which we had a small number of observations. The resultant equations were useful nonbiased predictive models for the estimation of speed (V) from length (L) and tail beat frequency (F) in each species (Table 4). The regression lines for these equations do not pass through the origin, however. They cut the abscissa before zero and consequently the intercept terms are negative. We pointed out previously that we believe the existence of a negative intercept in the raw data implied that the fish had a minimum swimming speed below which they cannot swim by beating only the caudal fin. Thus, to make the model more biologically meaningful we adjusted the elevation of the intercept function to correspond to the theoretical minimum swimming speed, V_0. (It should be remembered that the length-dependent slope of V_0 was about the same as the length coefficient, α_2, and it was this similarity that led us to use 2/3 as the intercept coefficient.)

To express the Case II model in terms of minimum speed we used V_0 estimated from Magnuson's equation to solve for a minimum tail beat frequency, F_0, and expressed the final relationship in the form

$$\frac{V - V_0}{L} = KF - F_0 .$$

In species other than *Trachurus* and *Carassius*, a theoretical estimate of V_0 was not possible and consequently we assumed V_0 was propor-

TABLE 4.—Swimming speed-tail beat frequency equations for seven species of fish.

Species	$V = \alpha_1 L^{2/3} + \alpha_2 L * F$	90% support-plane interval[1]
Trakis henles	$V = -1.39L^{2/3} + 0.93L * F$	$-2.71 < \alpha_1 < -0.01$ $0.77 < \alpha_2 < 1.09$ $s_{vf} = 2.49$
Trachurus symmetricus	$V = -2.50L^{2/3} + 0.83L * F$	$-3.28 < \alpha_1 < -1.59$ $0.78 < \alpha_2 < 0.88$ $s_{vf} = 12.39$
Scomber japonicus[2]	$V = -2.20L^{2/3} + 0.82L * F$	$-3.18 < \alpha_1 < -1.13$ $0.76 < \alpha_2 < 0.88$ $s_{vf} = 12.31$
Leuciscus leuciscus	$V = -1.58L^{2/3} + 0.74L * F$	$-2.21 < \alpha_1 < -0.86$ $0.71 < \alpha_2 < 0.78$ $s_{vf} = 7.78$
Carassius auratus	$V = -0.66L^{2/3} + 0.66L * F$	$-1.47 < \alpha_1 < 0.17$ $0.61 < \alpha_2 < 0.74$ $s_{vf} = 6.09$
Salmo gairdneri	$V = -1.28L^{2/3} + 0.64L * F$	$-2.63 < \alpha_1 < 0.13$ $0.59 < \alpha_2 < 0.69$ $s_{vf} = 17.72$
Sardinops sagax	$V = 0.49L^{2/3} + 0.50L * F$	$-1.61 < \alpha_1 < 2.58$ $0.39 < \alpha_2 < 0.62$ $s_{vf} = 5.77$

[1] Simultaneous confidence intervals for all parameters (Conway, Glass, and Wilcox, 1970).
[2] One deviant fish omitted, if fish included $V = -0.53L^{2/3} + 0.66L * F$, and $s_{vf} = 21.31$.

tional to $L^{2/3}$. The elevation of the line relating V_0 to length for a species was estimated by assuming that the lowest observed speed fell on that line. For *Trachurus* and *Carassius* we recalculated the elevation for a slope of 2/3.

Our estimates of V_0 and F_0 were not definitive. For *Trachurus* no fish were tested at speeds close to the theoretical minimum. Our estimates based on the theoretical minimum speed were closest to the observed minimum speeds in fish 9.4 cm total length and larger (Table 2) because in these larger fish the test speeds were sufficiently low for fish to swim with pectoral fins fully extended, an event that occurs near the minimum swimming speed (Magnuson, 1970). For *Carassius* the agreement between the theoretical estimate of minimum speed and observed minimum speeds was better (Table 5). The explanation for this is that the techniques used by Bainbridge (1958) permitted estimates at much lower speeds than the one we used. These data clearly show that in neither *Trachurus* (Table 2) nor *Carassius* (Table 5) was either V_0 or F_0 seriously overestimated. We feel that our estimates for these two species were reliable.

The fit to the general equation was good in all species (Figure 5, Table 6). The intercept for the regression line did not differ from zero and the scatter at low velocities was less than it was when no intercept correction was used (see figures in Bainbridge (1958) for comparison). The regression coefficient, K, in our equation differed among species. For the five species for which significant data were available, it was the highest in *Trachurus* and lowest in *Salmo*. Since amplitude was a constant, these results implied that the speed output per beat of the caudal fin was greater in *Trachurus* and *Scomber* than it was in *Salmo* and *Carassius*. In *Scomber*, the coefficient, K, may be uncertain because the values of one of the five fish tested deviated considerably from the rest. In Figure 5, all of the values to the right of the regression line above

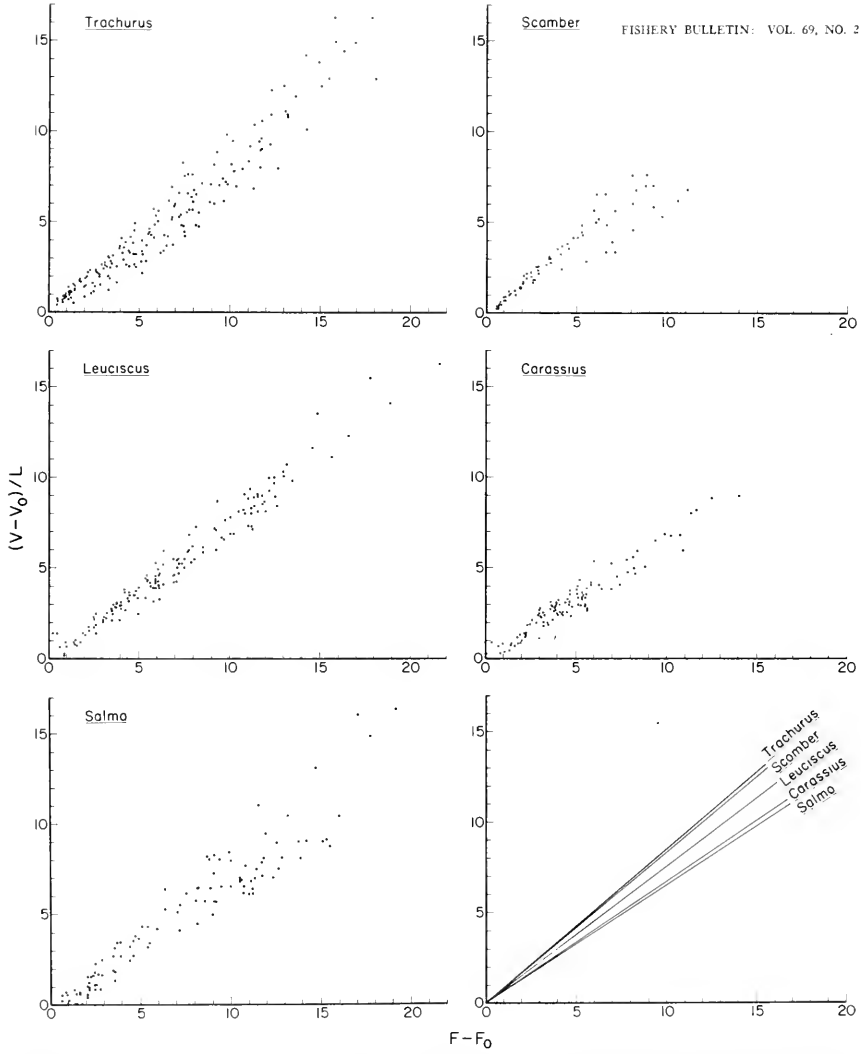

FIGURE 5.—Relationship between swimming speed corrected for minimum speed over length and tail beat frequency corrected for minimum frequency for *Trachurus* and *Scomber* from the present data, and for *Leuciscus*, *Salmo*, and *Carassius* from Bainbridge (1958). Graph at lower right shows individual regression lines for all above species, equations for lines are given in Table 6.

Table 5.—Slopes for the velocity-frequency relationship for individual *Carassius* studied by Bainbridge (1958) when the general relationship is slope $= 0.68L$; estimated minimum speed when $V_0 = 0.81L^{2/3}$; observed minimum swimming speed (V_{obs}); the tail beat frequency F_0 estimated by substitution of V_0 into the corrected slope equation; and the lowest observed tail beat frequency (F_{obs}).

Length (cm)	$b = 0.66L$	$V_0 = 0.81L^{2/3}$	[1]V_{obs}	$F_0 = 2.22L^{-1/3}$	F_{obs}
4.6	3.04	2.25	3.50	1.34	1.30
7.0	4.62	2.98	9.30	1.17	1.52
9 5	6.27	3.66	11.60	1.04	2 03
15.2	10.03	5.01	33.70	0.90	3.38
22.5	14.85	6.52	13.70	0.78	1.63

[1] Data from Bainbridge (1958).

TABLE 6.—Minimum speed (V_0), minimum tail beat frequency (F_0), the coefficient K in equation $V - V_0 = L(KF - F_0)$ arranged in order of K.

Species	N	V_0	F_0	K
Triakis henlei	6	$0.15L^{2/3}$	$1.66L^{-1/3}$	0.93
Trachurus symmetricus	176	[1]$0.80L^{2/3}$	$3.98L^{-1/3}$	0.83
Scomber japonicus	[2]61	$1.31L^{2/3}$	$3.51L^{-1/3}$	0.82
Leuciscus leuciscus[3]	149	$0.67L^{2/3}$	$3.04L^{-1/3}$	0.74
Carassius auratus[3]	111	[1]$0.81L^{2/3}$	$2.22L^{-1/3}$	0.66
Salmo gairdneri[3]	109	$0.52L^{2/3}$	$2.81L^{-1/3}$	0.64
Sardinops sagax	9	$2.23L^{2/3}$	$3.48L^{-1/3}$	0.50

[1] V_0 theoretical estimate based an equation af Magnuson (1970).
[2] One deviant fish omitted; if fish included, $N = 74$, $K = 0.66$.
[3] Originol data from Bainbridge (1958).

4 beats/sec on the abscissa were from this single deviant fish. If the deviant fish is included $K = 0.66$, but if not, $K = 0.82$. We are inclined to use $K = 0.82$ because the values for the four fish were very similar and the protocol indicated that the deviant fish may have been overly fatigued when tested. *Triakis* appears to have a relatively high coefficient but not too much significance can be attached to the exact value for *Triakis* or for *Sardinops* because these were based on so few measurements.

In sum, the speed-tail beat equation (Case II) —Table 6—was biologically as well as statistically relevant, was sensitive to specific differences in swimming behavior, provided an unbiased correction for length, and made possible a more accurate estimation of swimming speed from tail beat frequency than heretofore has been possible.

TAIL BEAT AMPLITUDE

We pointed out previously that tail beat amplitude was a constant and was directly proportional to length and consequently the size coefficients for amplitude are probably the same as those for length. Thus amplitude (A) in centimeters can be substituted for length in the original Case II equation $V = \alpha_1 A^{2/3} + \alpha_3 L * F$. When this was done for *Trachurus* using all individual amplitude values ($N = 176$), we obtained the equation; $V = -6.5767A^{2/3} + 3.5637A * F$. The tail beat amplitude coefficient may be also estimated by substitution of the amplitude-length relationship for *Trachurus* ($A = 0.23177L$), into the Case II equation.

The tail beat amplitude data collected by Bainbridge (1958) were insufficient for specific estimates of an amplitude coefficient. The mean amplitudes for each of the fish we studied and for each of those studied by Bainbridge were nearly the same, when adjusted for body length. Variation within a species was as great as that between species (Figure 6). The relationship

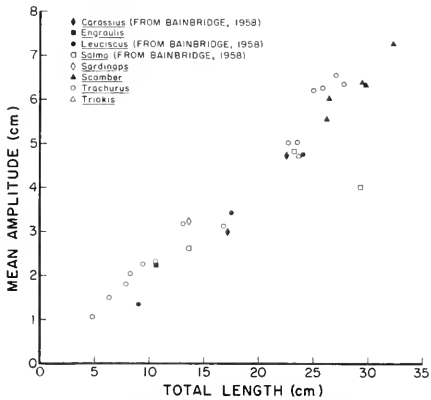

FIGURE 6.—Relationship between mean tail beat amplitude and length for every fish we studied and all those studied by Bainbridge (1958), $A = 0.21L$.

between mean tail beat amplitude and length for all species combined was $A = 0.21L$.

DISCUSSION

In all previous studies speed was divided by length then related to tail beat frequency. In our data when speed was converted to body lengths per second the relationship between speed and frequency was nearly identical to that given by Bainbridge (1958) for *Carassius*, *Leuciscus*, and *Salmo*. The confidence intervals for the slopes in the speed-frequency relationship in *Euthynnus* and *Thunnus* (Yuen, 1966) and in *Sarda* (Magnuson and Prescott, 1966) overlap the slope in the Bainbridge equation and the ones for *Trachurus* and *Scomber* when the body length conversion is used. Thus, when speed is in body lengths per second, the relationship between it and frequency is about the same in all fish studied to date from goldfish to mackerel and is adequately described by the Bainbridge equation $V/L = bf$. Thus the Bainbridge equation provides a description of the average relationship for fish in general but little significance can be attached to specific differences in slope. If more than a rough estimate of speed is required or if specific differences are important, or if estimates are needed near the minimum swimming speed it would be necessary to use the equation developed in this study.

Bainbridge (1958) concluded from his data that the frequency-speed relationship was curvilinear below a frequency of about 5 beats/sec because fish modulated their tail beat amplitude. His evidence for this conclusion was that in some fish amplitude appeared to decrease at lower frequencies, and that the distance per beat, calculated by dividing speed by frequency, declined at frequencies below 5 beats/sec but was constant above that frequency. His evidence for amplitude modulation at low speeds was weak. In the three *Salmo* studied no trend existed; in *Carassius* he suggested there might be a decrease in amplitude in one of the two fish studied, and in one of the two *Leuciscus* studied a trend existed slightly stronger than the one in *Carassius*. In sum, the evidence for a decline in amplitude measurements was based on possible trends in two of the seven fish studied. Two fish could easily give a misleading picture of the general trend in the data, especially when the variability in amplitude measurements are considered. In our studies we measured the tail beat amplitude in every fish at all possible speed levels and no evidence existed for a consistent change in amplitude with speed.

In Bainbridge's data the departure of distance traveled per beat from a constant at low frequencies was caused by the division of speed by frequency. Had the line relating frequency to speed passed through the origin, no bias would have existed but because the line intersected the abscissa at about 1 beat/sec division by frequency produced an artificial curvilinear trend at lower frequencies. We produced the same trend in distance per beat in our data by dividing speed by frequency but the trend was eliminated when a correction for the intercept was used. Thus the curvilinear trend in distance per beat in Bainbridge's data was an artifact caused by the method of calculation and consequently distance per beat was a constant at all frequencies. In addition, the apparent nonlinearity below 5 beats/sec in his graphs relating speed divided by length to frequency was also the result of the same intercept problem. Therefore, no evidence exists for consistent amplitude modulation at any speed range and speed appears to be related only to tail beat frequency and length in the species studied by Bainbridge (1958) as well as in the ones we studied. We concluded that during steady swimming at any speed, tail beat amplitude is a constant proportion of body length of the order of 0.21 L.

That the mean amplitude during steady swimming was constant does not mean that amplitude is not modulated under certain conditions. It is widely known that fish modulate tail beat amplitude when they accelerate (Gray, 1968). Further, we had the impression that some of the variability in the speed-frequency relationship was caused by differences in amplitude. These differences were infrequent and irregular in occurrence and consequently we were not able to evaluate them statistically. We are inclined to believe, however, that fish occasionally made minor adjustments in amplitude and frequency over the entire range of speeds, but these adjust-

ments were merely individual deviations from the general relationship we have described.

We do not wish to detract from the original and important contribution of Bainbridge (1958), by emphasis on the differences between his and our conclusions. His basic conclusions and equations were not greatly different from our own. We were able to examine more closely the form of the relationships he described because of a larger sample size made possible by the availability of automatic film analysis equipment and because of the existence of his data in the literature.

The question of species-specific size effects remains unresolved. In our general model a good fit was obtained in seven species when the minimum stalling speed was proportional to $L^{2/3}$, the frequency at this minimum speed was proportional to $L^{-1/3}$, and the slope coefficient was proportional to $L^{1.0}$. A comparative study on speed-related size effects in fishes would certainly be of value.

It also remains to be resolved whether or not it was appropriate to apply the minimum swimming speed equation developed by Magnuson (1970) for *Euthynnus affinis*, a fish that lacks a swim bladder, to such a broad assortment of species. The equation implies a functional relationship between minimum speed and hydrostatic equilibrium and implies existence of negative buoyancy at minimum speeds. We do not know if these relationships exist in all species; nevertheless his equation did provide a reasonable estimate for minimum speed and it functioned well in our equation.

The relationship between swimming speed and tail beat frequency we have described could be used in any application where it is necessary to measure swimming speeds of fish. For example, a sonic internal tag could be developed that telemetered tail beat frequency and thus the speed of free-swimming fish could be monitored continuously over extended periods.

The tail beat frequency-speed relationship could be used for size or species identification using Continuous Transmission Frequency Modulated sonar as suggested by Hester (1967). The increase of speed with frequency (our K value) varied from species to species and thus might be used for identification. If size were known, the minimum observed velocity would provide additional information for identification. Alternatively, if the species were known, minimum (or maximum) speed would provide an indication of size. The equation could also be used to estimate size from tail beat amplitude, but caution should be exercised because in our study amplitude was not modulated and consequently, we do not know whether or not speed and tail beat amplitude are linearly related within an individual.

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SUSTAINED SPEED OF JACK MACKEREL, *Trachurus symmetricus*

JOHN R. HUNTER[1]

ABSTRACT

Jack mackerel, *Trachurus symmetricus*, were forced to swim for up to 6 hr at various speeds in an activity chamber. The probit estimate for the swimming speed at which 50% of *Trachurus* would fatigue during 6 hr was 93.4 cm/sec (8.4 *L*/sec) for fish 10.0 to 11.9 cm and was 22.4 $L^{0.6}$/sec for fish 9.0 to 17.6 cm where *L* is the total length of the fish in centimeters. At higher speeds, *Trachurus*, 15 cm, swam for 3 min at 160 cm/sec or 10 *L*/sec. The swimming speed at which 50% fatigued declined exponentially with time for about the first 22 min of swimming and thereafter declined linearly with time. The possible significance of the time-speed relationship for *Trachurus* is discussed.

Although a substantial literature on the swimming speed of fishes exists (see Bainbridge, 1958; Gray, 1968), few reliable estimates of maximum sustained speed exist. Much of the literature on swimming speed of fishes is concerned with estimates of maximum speed or burst speed, that is, speeds that can be maintained for only a few minutes or less. A sustained speed implies, on the other hand, that the animal is capable of swimming at that speed for hours. For example, Brett (1967) recommended a minimum of 200 min for a fixed sustained speed test. Fairly wide agreement exists that 2 to 3 *L*/sec can be maintained for an hour or more and salmonids and herring seem capable of sustaining 3 to 4 *L*/sec for such periods (Blaxter, 1969). These conclusions were drawn primarily from studies of freshwater fish and salmon; no estimates of maximum sustained speeds have been made for fast-swimming pelagic marine forms. The object of this study was to determine the sustained speed threshhold of jack mackerel, *Trachurus symmetricus*, a pelagic marine fish of commercial importance. The body form and musculature of *Trachurus* appear to be designed for greater hydrodynamic efficiency at high speeds than other species heretofore studied. In *Trachurus*, lateral musculature is concentrated in the anterior portion of the trunk, and inserts by tendons on a small deeply forked caudal fin.

In addition to the interest in comparing the sustained speed capabilities of *Trachurus* with that of fish with other body forms, sustained speed data have significance in prediction of migratory capabilities and physiological limits.

APPARATUS AND METHODS

The apparatus used in the experiments was an activity chamber provided with a water current of various calibrated speeds. The apparatus was the same as the one described and figured by Hunter and Zweifel (1971) in this issue except that a port was provided in the transparent hatch of the swimming chamber so that fatigued fish could be removed by hand from the downstream screen without reducing the flow in the chamber. The error in estimating the water speed in the swimming chamber did not exceed 10% and it was assumed that the fish were swimming at the estimated speed.

The experimental design was essentially the same as that used by Brett (1967) for determining the sustained speed threshhold for sockeye salmon, *Oncorhynchus nerka*. Fifty-five groups of five *Trachurus* (9.0 to 17.6 cm total length, mean = 12.43 ± 0.11 cm) were subjected to a fixed speed of 38 to 160 cm/sec for 360 min or longer after an introductory period of about 30 min at a low speed. A time-lapse camera photographed the fish at 1-min intervals and the time to fatigue for each fish was determined from the photographs. The temperature of the water in the activity chamber and in the holding

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tank (a plastic swimming pool 15 ft in diameter) was maintained by a temperature regulation system at about 18.5° C. The mean test temperature was 18.48 ± 0.03° C. The fish were captured near Santa Catalina Island, Calif., on 12 September 1969. Tests began 2 weeks later and ended on 21 November 1969. Fish were fed an abundant ration of chopped squid, anchovies, and frozen brine shrimp. Probit analysis, a statistical technique first applied to sustained speed data by Brett (1967), was used to estimate sustained speed threshholds.

Variability in length posed a problem in the analysis. Although all fish were from the same school, differences in length existed; also the fish grew in the course of the study. These differences were insufficient, however, to determine the form of the relationship between length and sustained speed. In general, the relationship between length and sustained speed for other species (Bainbridge, 1962; Brett, 1965), theoretical considerations (Gray, 1968; Fry and Cox, 1970), and relationships between length and other swimming capabilities (Magnuson, 1970), indicate that speed is proportional to a fractional power of length equal to about $L^{0.6 \pm 0.1}$. In addition, the minimum swimming speed of *Trachurus* was proportional to $L^{0.6}$ when estimated from Magnuson's equation (Hunter and Zweifel, 1971). In light of the above evidence it seemed preferable to use 0.6 as the coefficient of length, although unity has been commonly employed in cases where length coefficients were unknown. As an alternative to this procedure I also estimated the percent fatigued at different speeds in centimeters per second and in body lengths per second for a narrow length range (10.0 to 11.9 cm total length) where the effect of differences in length would be negligible.

RESULTS

Within a few minutes after *Trachurus* were placed in the swimming compartment they became quiescent, swam steadily, and remained in about the same position in the compartment throughout the test or until they became fatigued and fell against the rear screen. This was in contrast to some other species which did not swim steadily, but swerved and oscillated from side to side.

The relationship between water speed and percent fatigue had the normal sigmoid form of a dosage response curve (Finney, 1952). Probit estimate of the applied water speed at which 50% fatigue occurred in 360 min of swimming and the 95% confidence limits were 94.40 ± 5.15 cm/sec for *Trachurus* 10.0 to 11.9 cm total length, $N = 127$ (Figure 1, Table 1). Thus,

TABLE 1.—Swimming endurance of *Trachurus symmetricus* in cm/sec and in $L^{0.6}$/sec.

Length 10.0-11.9 cm					Length 9.0-17.6 cm		
Speed cm/sec	N	Percent fatigued	Length Mean	Length SD	Speed[1] $L^{0.6}$/sec	N	Percent fatigued
71	8	0	10.69	0.61	15.9	3	0
78	16	13	10.71	0.47	16.9	10	0
85	16	31	10.84	0.67	17.8	14	0
92	14	50	10.97	0.54	18.7	17	6
99	21	62	11.23	0.43	19.7	18	17
106	21	76	10.85	0.56	20.6	23	39
113	15	100	11.41	0.42	21.5	39	31
120	8	100	11.27	0.45	22.5	42	67
138	8	100	11.20	0.51	23.4	19	58
Total	127				24.3	20	60
					25.3	29	72
					26.2	19	100
					27.2	9	100
					28.1	4	100
					29.0	3	100
					30.0	1	100
					30.9	3	100
					31.8	7	100
					32.8	10	100
					33.7	4	100
					Total	294	

[1] Total speed range divided into 20 equal intervals; speeds listed are midpoints of those intervals.

50% of *Trachurus* in this length range could be expected to sustain a speed of about 8.4 L/sec or 22.1 $L^{0.6}$/sec for 360 min. For all *Trachurus* ($N = 294$) the water speed at which 50% fatigue occurred after 360 min of continuous swimming and the 95% confidence intervals were 22.4 ± 1.2 $L^{0.6}$/sec. The first estimate, based on a narrow length range, and the second one, based on all data, were reasonably close. On the other hand, when all data were in the form $V/L^{1.0}$ the 50% threshold was 9.34 L/sec which is higher than the preceding estimates. Inspection of these data, however, showed that the coefficient for length clearly was less than one and that use of unity biased the estimate.

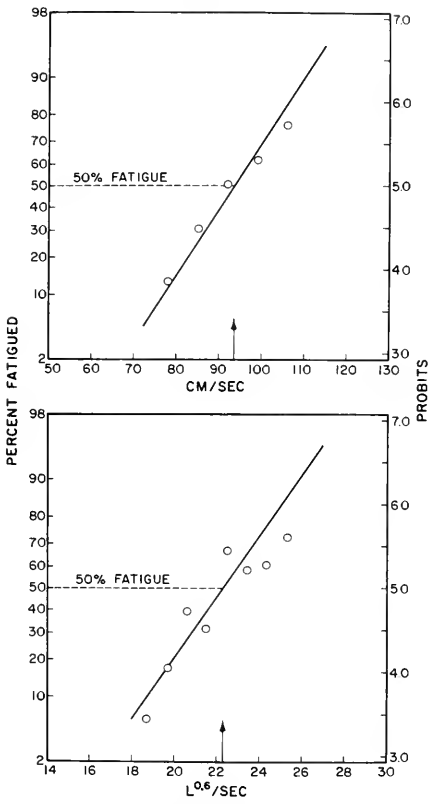

FIGURE 1.—Probit lines for sustained speed threshold for 6 hr of forced swimming at 18.5° C in juvenile *Trachurus symmetricus*. Upper panel, range fish length 10.0 to 11.9 cm $N = 127$, probit $= 0.077X - 2.238$; lower panel, range fish length 9.0 to 17.6 cm, $N = 294$, speed expressed in $L^{0.6}$/sec where L is the total length of the fish, probit $= 0.355X - 2.958$.

To determine the form of the relationship between the duration of the swimming period and the ability to maintain a certain speed, probit estimates of speed for five levels of fatigue were made for swimming periods varying from 10 to 360 min. The form of the relationship was about the same for all fatigue levels; speed estimates declined exponentially with time for short swimming periods and linearly with time for longer ones (Figure 2). The point

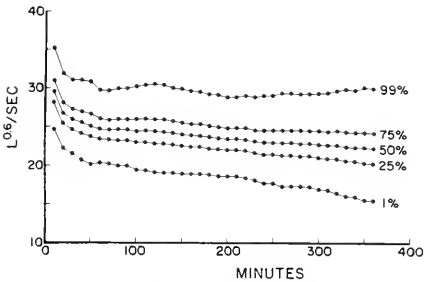

FIGURE 2.—Relation between speed in $L^{0.6}$/sec and the time it can be sustained for 1, 25, 50, 75, and 99 percent fatigue levels in *Trachurus symmetricus*. Estimates of speed for each fatigue level made at 10-min intervals of cumulated time.

of inflection from the exponential to the linear relationship was examined in detail for the 50% fatigue level. Probit estimates of the speed at which 50% of the fish fatigued were made for 2-min intervals of swimming cumulated over the first 100 min of observation. The data were plotted on semilog paper and a line fit by eye to the exponential function. The point of inflection appears to occur at about 22 min (Figure 3). Thus, speed at which 50% fatigued and the duration of the swimming period were exponentially related for durations up to about 22 min and were linearly related for longer periods of swimming.

The performance of juvenile *Trachurus* at high speed was of interest. Fifteen fish 14.6 cm mean total length (range = 13.4 to 16.6 cm) swam at the highest speed used in the study (160 cm/sec) for 2 to 6 min, mean time 3.4 min. Thus, *Trachurus* 15 cm total length were able to swim for about 3 min at about 10 L/sec or about 32 $L^{0.6}$/sec. A slightly higher level of

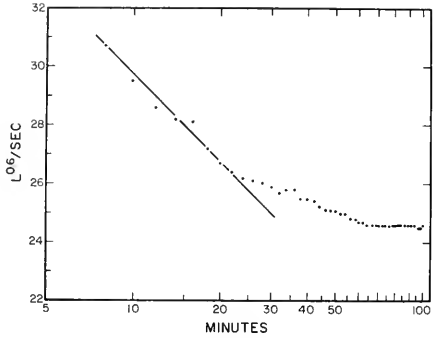

FIGURE 3.—Relation between speed at which 50% *Trachurus* fatigued and the duration of the swimming period. Duration of swimming period in minutes plotted on log scale to show exponential trend; line fit by eye. Speed estimates made at 2-min intervals of cumulated time over the first 100 min of swimming.

performance in length per second is obtained if we consider smaller fish. For example, fish of mean length 11.2 cm (length range 10.4 to 11.9 cm, $N = 8$) swam for 3 to 5 min (mean = 4.5 min) at 139 cm/sec or about 12 L/sec. This difference between large and small fish becomes negligible if 0.6 is used as a coefficient of length instead of unity because, as was pointed out previously, the length coefficient for *Trachurus* appears to be less than 1.

DISCUSSION

The exponential decline in swimming speed with time in fish is well documented; see for example Bainbridge (1960), Brett (1967), and Blaxter (1969). The general form of the relation between time and swimming speed in other fish resembles that for *Trachurus* although the speeds and endurance times are different in *Trachurus*. The physiological mechanisms responsible for the exponential relationship between swimming speed and endurance are generally believed to be the limited enery stores in the muscle, the rate these stores can be replaced and the rate catabolites are removed from the muscle (Bainbridge, 1960). A study by Pritchard, Hunter, and Lasker (1971) in this issue has provided an explanation for the form of the speed-time relationship in *Trachurus*. Pritchard et al. found that at speeds where an exponential relationship exists between time and speed the principal cause of failure of *Trachurus* was most likely the depletion of glycogen in the white muscle. On the other hand, fish that failed at speeds near the 6-hr 50% threshold, where a linear relationship exists between speed and time, had depleted not only the glycogen in the white muscle but that in the red muscle and liver as well. Thus, in *Trachurus* the form of the time-speed relationship could be explained on the basis of the extent of glycogen reserves available for locomotion and the time required to mobilize them from sites other than the white muscle. An exponential relationship between speed and time could be produced when the speeds are so high that the glycogen supply would be limited almost entirely to the white muscle because the supply in the white muscle would be used up and the fish would fail before significant amounts of glycogen could be mobilized from other sources. A linear relationship could exist where swimming speeds are sufficiently low that reserves in the white muscle could not be depleted before other sources in the red muscle and the liver are mobilized. We have, on one hand, a high rate of consumption using a more limited supply of fuel which could lead to an exponential relationship between speed and time and, on the other hand, a much lower rate of consumption using a relatively much larger fuel supply which could produce a linear relationship with time. An exponential relationship between energy consumption and swimming speed would enhance these effects.

Let us now consider the significance of the 6-hr sustained speed threshold determined for *Trachurus*. When compared with other determinations, this threshold appears to be unique because of different physiological mechanisms and because it is higher than those estimated for other fish. *Trachurus* at threshold speed appeared to use glycogen as fuel, white muscle for locomotion and maintained a high lactic acid

level in the muscle (Pritchard et al., 1971). These results are inconsistent with the conclusion that at sustained cruising speeds, fish use lipid metabolism to drive red muscle (Bone, 1966; Gordon, 1968; Blaxter, 1969) and that no oxygen debt is incurred (Brett, 1963). Reliance on glycogen as the principal fuel probably severely limits the time a speed can be maintained as compared with one where lipid metabolism is used exclusively. Thus the biochemical evidence indicates that the 6-hr speed threshold for *Trachurus* probably could be maintained only for a period of hours or perhaps days but certainly not weeks as one would expect if fat were used as fuel. The 6-hr threshold was also considerably above sustained speed thresholds for other fish where presumably fat may be employed as fuel. Brett (1967), in a study directly comparable with the current one, found the 50% fatigue time for sockeye salmon was 4 L/sec (about 11.3 $L^{0.6}$) whereas for comparable size jack mackerel it would be about 7.6 L/sec or 22.0 $L^{0.6}$. Other less comparable data give sustained or cruising speeds in the range of 3 to 4 L/sec (Blaxter, 1969). Thus, *Trachurus* has special physiological and structural adaptations that permit swimming for periods of hours at elevated speeds and it was the threshold for this swimming behavior that was measured. Other fishes, especially the scombroid fishes, may have similar abilities. For example, skipjack tuna can swim at 8 knots, or about 43 $L^{0.6}$, for over an hour (Commercial Fisheries Review, 1969) and yellowfin tuna and skipjack tuna have higher levels of white muscle glycogen than many other species of fish (Barrett and Connor, 1964).

It seems possible another speed threshold may exist for *Trachurus* below the present one where fat is the principal fuel, only red muscle is used for locomotion, and swimming can be maintained almost indefinitely. It would not be surprising if this lower threshold were closer to those determined for other fishes.

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THE TRANSPLANTING AND SURVIVAL OF TURTLE GRASS, *Thalassia testudinum*, IN BOCA CIEGA BAY, FLORIDA[1]

JOHN A. KELLY, JR., CHARLES M. FUSS, JR., AND JOHN R. HALL[2]

ABSTRACT

Turtle grass was transplanted to an unvegetated, dredged canal and a hand-cleared portion of a flourishing grass bed. Complete or partial success was attained in 7 of 14 methods used. The best method, in which short-shoots (rhizomes removed) were dipped in a solution of plant hormone (Naphthalene Acetic Acid) and attached to construction rods for transplanting, was 100% successful and may be suitable for general application.

Turtle grass, *Thalassia testudinum*, and other marine grasses are an invaluable asset to the marine ecosystem. They are primary producers and form an essential ecological niche in which a great number and variety of species find food and shelter. They are also important agents in the control of substrate erosion and the depositions of sediments (Stephens, 1966).

Uncontrolled dredging and filling of submerged lands have destroyed many turtle grass beds and their dependent fauna, some of which are economically important. An immediate need exists not only for sharply restricting further destruction of sea grass beds but also for replacing lost beds. One method of replacing them may be by transplanting sea grasses to areas that are suitable for their growth or to areas that are made favorable by soundly planned engineering (Phillips, 1960; Strawn, 1961). Areas surrounding spoil banks and finger-fill canals (dredged canals between filled land masses) would be suitable if they were constructed to supply zones of optimum depth for growth of marine grasses.

Unsuccessful earlier attempts to transplant turtle grass in Tampa Bay showed that the main problem was erosion by tidal currents. Turtle grass is buoyant, and new transplants tend to work free of the sediments and float to the surface when disturbed by water movement (Phillips, personal communication).[3] Another marine plant, eelgrass (*Zostera marina*), was transplanted successfully on the coast of Washington by Phillips (1967) and in the Aleutian Islands by Jones[4] and McRoy[5] (personal communication), but details on methods are not yet published. Successful growth of turtle grass under artificial conditions (Fuss and Kelly, 1969) led us to attempt transplanting it from one field location to another as described in the present paper.

Turtle grass spreads vegetatively by creeping rhizomes (long-shoots) buried in the substrate (Figure 1). Work by Tomlinson and Vargo (1966) showed that this growth is dependent entirely upon the vigorous activity of meristematic tissue in the apexes of rhizomes. The apex is also the only source of short-shoots (erect lateral branches) that develop from buds at this site. In the Miami area (Phillips, 1960) and tropical parts of its range, the plants also reproduce by flowering. Tampa Bay, however, is near the northern limit of the flowering capability of *Thalassia* (Phillips, 1960); thus, we

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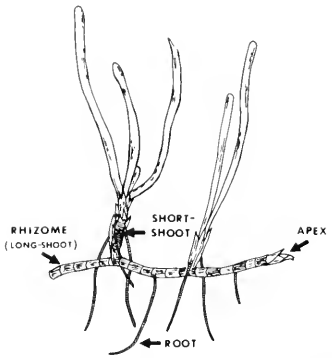

FIGURE 1.—External features of *Thalassia testudinum*.

confined our restoration studies to the transplantation of adult plants. This paper describes the procedures for and results of transplantation of turtle grass into modified environments.

DESCRIPTION OF TRANSPLANT SITES

All experiments took place in the southern end of Boca Ciega Bay, Fla., an elongate coastal lagoon joined to Tampa Bay and separated from the Gulf of Mexico by a line of barrier islands (Figure 2). The area it encompasses is a paramount example of grass bed destruction by hydraulic engineering (Hutton et al., 1956; Phillips, 1960; Taylor and Saloman, 1968).

A rectangular area 8.2 by 21 m (27 by 7 ft) in a large turtle grass bed was cleared by hand to serve as the control site. Two other transplant areas of the same size were in two adjacent finger-fill canals in a large land-fill development.

Construction of houses had not begun along the canals selected, and none was built during the experiments. Boating in the canals was light, and during periodic inspections we saw no disturbance of the plants directly attributable to man.

Sediments from transplant sites were analyzed by particle size. A sample from the control site was 95.5% sand (>62.5μ) and 4.5% silt and clay (<62.5μ) on a dry weight basis. At the planted areas of the finger-fill canals, sediments averaged 98.6% sand and 1.4% silt and clay. No analysis of the carbonate fraction was made for these samples; however, all sites had shell fragments, which appeared to be more abundant in the canals than at the control site.

MATERIAL AND METHODS

The work was divided into two phases: Phase I extended from July 1966 through August 1967 and phase II from April through October 1967. In phase I, methods of deflecting and reducing the force of tidal currents and waves in the vicinity of transplants and of anchoring new transplants in the substrate were tested. Concrete building blocks were laid in parallel rows at both transplant sites to form enclosed areas for sheltering new transplants against the forces of moving water (Figure 3). Plugs of grass approximately 8 inches square (20 × 20 cm)

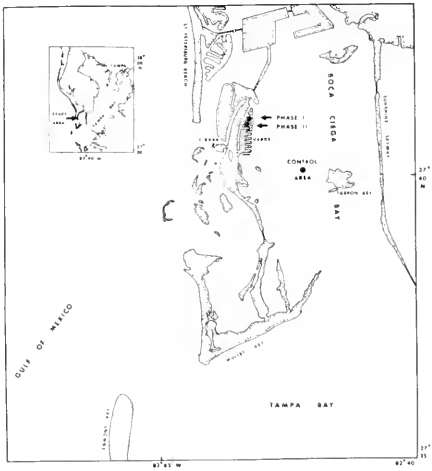

FIGURE 2.—Locations of experiments (phases I and II, and control area).

and containing four to five short-shoots were dug from natural beds adjacent to the control site. Three methods of transporting and anchoring the plugs were tried: (1) placing them in tin cans, (2) balling them in burlap, and (3) temporarily bagging the roots and rhizomes in polyethylene, which was removed just before planting.

A total of 120 plugs was transplanted — 60 at the control site and 60 at the finger-fill canal. At both locations, 30 were placed inside and 30 outside of enclosures. Each group of 30 plugs was planted three ways: 10 in cans, 10 in burlap, and 10 unanchored (Figure 3).

Phase II consisted of testing additional anchoring devices to hold individual sprigs of turtle grass in the substrate of the finger-fill canal. The devices were cast iron, 2-inch (5.1-cm) pipe, brick, and construction rod. Sprigs used in this study were single short-shoots with leaves, many roots, and with or without a portion of the parent rhizome. Also tested in phase II was the plant hormone, NAPH (Naphthalene Acetic Acid),[6] which is used for rooting grass stolons and plant cuttings.

Sixty sprigs, obtained from the same natural bed as the plugs in phase I, were washed and prepared for the experiment by breaking entire rhizomes from some, breaking only the apexes of rhizomes from others, and leaving the rhizomes attached and entire on others. Half of the sprigs were placed in a 10% solution of NAPH in seawater for 1 hr. The other half were left untreated. The sprigs were planted in groups to test various combinations of treatment and nontreatment with NAPH, presence and absence of apexes of rhizomes, presence and absence of rhizomes, and types of anchors (Figure 4).

Sprigs anchored with construction rod had no rhizomes. Sprigs anchored with pipe had rhizomes that were buried in hand-dug holes; whereas, sprigs anchored with brick were simply placed on the surface of the substrate and their rhizomes held in contact with the sediment by the weight of the brick.

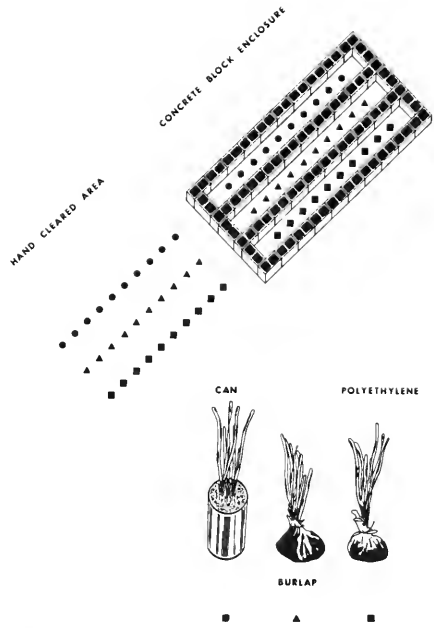

FIGURE 3.—Details of concrete-block wave and current barriers, tin-can anchors for plugs, and placement of transplants at planting sites.

RESULTS

Transplants were considered successful if they established themselves in the new environment and exhibited new rhizome growth (Figure 5). Individual sprigs met these criteria if short-shoots appeared healthy, had new roots, and had either given rise to a new rhizome or were still part of an old long-shoot with an active apex. Plug transplants (phase I) were considered successful if only one of the short-shoots met the above criteria.

[6] Manufactured by Nutri-Sol Chemical Company, Tampa, Fla. 33609. References to trade names in this publication do not imply endorsement of commercial products.

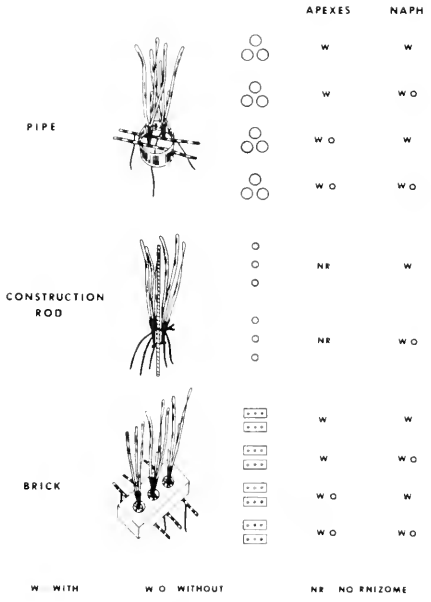

FIGURE 4.—Details and treatment of sprigs anchored by pipe, brick, and construction rod; and placement of transplants at planting sites.

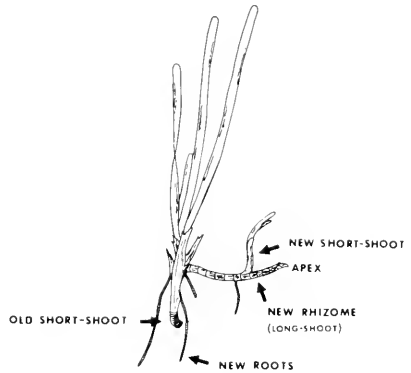

FIGURE 5.—New rhizome, root, and short-root growth on a sprig of turtle grass transplanted without an intact rhizome apex.

Plugs in phase I planted July 1966 were removed from the sites late in August 1967, approximately 13 months after planting. Six of the 40 transplants at the canal area and 16 of the 40 at the control area were successful (Table 1).

Planting individual sprigs of turtle grass in phase II yielded similar results. Of the 60 sprigs planted in the second canal in April 1967 and removed in mid-October 1967 (about 6 months after they were transplanted), 11 were successful (Table 2).

Successful new growth of rhizomes represented 15 and 18% of the number of transplants made in the finger-fill canal in phases I and II, respectively, and 40% of the transplants made in the control bed (Tables 1 and 2). Transplant attempts made with burlap in phase I were not included in the above percentages because all failed within 1 month.

EROSION CONTROL

Results of planting plugs within concrete-block enclosures were completely different in the finger-fill canal and grass bed locations (Table 1). In the canal the only successful transplants grew within the protection of the block enclosures; none planted without this protection survived, and most of the latter failed within the first 6 months of the experiment. Most of the successful plugs placed in the control area were planted outside the concrete blocks and, throughout the study, appeared to be in better condition than those inside the enclosures. The enclosures fulfilled their purpose in the canal but appeared detrimental in the control area. In the latter region, surrounding grass beds apparently provided sufficient protection from water movement. Enclosures in

TABLE 1.—Surviving transplants, successful transplants, and seasonal mortality of transplanted plugs of *Thalassia* in the finger-fill canal and the control site, phase I, July 1966 through August 1967.

Method of protecting and anchoring	Transplants	Surviving plants			Mortality			
		Unsuccessful[1]	Successful[2]		Fall	Winter	Spring	Summer
	No.	*No.*	*No.*	*%*	*No.*	*No.*	*No.*	*No.*
			Finger-fill canal site					
Inside concrete block enclosures:								
Anchored in cans	10	0	3	30	3	4	0	0
Unanchored	10	0	3	30	2	4	0	1
Total	20	0	6	30	5	8	0	1
Outside concrete block enclosures:								
Anchored in cans	10	0	0	0	9	1	0	0
Unanchored	10	0	0	0	10	0	0	0
Total	20	0	0	0	19	1	0	0
Grand total	40	0	6	15	24	9	0	1
			Control site					
Inside concrete block enclosures:								
Anchored in cans	10	1	3	30	5	1	0	0
Unanchored	10	2	0	0	4	1	2	1
Total	20	3	3	15	9	2	2	1
Outside concrete block enclosures:								
Anchored in cans	10	1	7	70	0	1	0	1
Unanchored	10	2	6	60	1	1	0	0
Total	20	3	13	65	1	2	0	1
Grand total	40	6	16	40	10	4	2	2

[1] Transplants survived but did not exhibit new rhizome growth.
[2] Transplants exhibited new rhizome growth.

the control area often filled with a heavy accumulation of light-robbing algae, dead grass, and other detritus, which quickly resulted in burial and death of the entire transplant.

ANCHORING METHODS FOR PLUGS

Of the plugs anchored with tin cans, 50% were successful at the control site, but only 15% in the canal (Table 1). None of the plugs planted in cans outside of the concrete-block enclosures survived. Cans were thus ineffective against currents unless used in conjunction with the concrete-block current barrier.

Plugs transported in polyethylene bags and then directly transplanted served to evaluate the effect of tin cans. We noted no adverse effects from the metal in the cans. The ratio of successes of unanchored to anchored transplants was 3:5.

The reasons for the rapid failure of plugs with roots and rhizomes wrapped in burlap is unknown. Possibly decomposition products of the decaying burlap, such as H_2S, or toxic chemicals in the material caused the plants to die.

ANCHORING METHODS FOR SPRIGS

In phase II sprigs were planted with added anchoring devices but without the aid of the wave and current barriers.

Construction rod was the most effective device used to anchor sprigs. It was the easiest to handle because all sprigs fixed to it were transplanted without rhizomes and were simply fastened to the rod with plastic-coated wire and inserted into hand-dug holes in the substrate. Of the 12 sprigs anchored with rod, only the 6 that had been treated with the hormone NAPH became established (Table 2). Sprigs that did not survive failed early in the experiment and simply disappeared, probably because they were dislodged by water movement in the canal before roots were developed.

TABLE 2.—Surviving transplants, successful transplants, and monthly mortality of transplanted sprigs of *Thalassia* in a finger-fill canal, phase II, April through October 1967.

Method of anchoring and treating	Transplants	Surviving plants			Mortality[1]				
		Unsuccessful[2]	Successful[3]		May	July	Aug.	Sept.	Oct.
	No.	*No.*	*No.*	*%*	*No.*	*No.*	*No.*	*No.*	*No.*
Pipe[4]									
With apexes, with NAPH	6	0	0	0	6	0	0	0	0
With apexes, without NAPH	6	0	0	0	0	6	0	0	0
Without apexes, with NAPH	6	2	2	33.3	0	0	0	2	0
Without apexes, without NAPH	6	5	1	16.7	0	0	0	0	0
Total	24	7	3	12.5	6	6	0	2	0
Brick[5]									
With apexes, with NAPH	6	4	2	33.3	0	0	0	0	0
With apexes, without NAPH	6	0	0	0	0	6	0	0	0
Without apexes, with NAPH	6	0	0	0	0	6	0	0	0
Without apexes, without NAPH	6	0	0	0	3	3	0	0	0
Total	24	4	2	8.3	3	15	0	0	0
Construction rod[6]									
With NAPH	6	0	6	100.0	0	0	0	0	0
Without NAPH	6	0	0	0	0	4	2	0	0
Total	12	0	6	50.0	0	4	2	2	0
Grand total	60	11	11	18.3	9	25	2	2	0

[1] Mortality not observed in June.
[2] Transplants survived but did not exhibit new rhizome growth.
[3] Transplants exhibited new rhizome growth.
[4] Rhizomes were buried; two sprigs per anchor.
[5] Rhizomes were not buried; three sprigs per anchor.
[6] Rhizomes were removed before planting; two sprigs per anchor.

Pipe and brick were poor anchors. The sprigs anchored with pipe were transplanted with their rhizomes and special care was required in burying them to avoid breakage. Almost half of the 24 sprigs held with pipe lived to the end of the experiment, but only 3 exhibited new rhizome growth (Table 2). Sprigs secured to the bottom with brick were not buried but were simply laid on the bottom and the substrate was scooped over them by hand. They were also transplanted with their rhizomes but were difficult to handle because of their tendency to slip out from underneath the brick before they were finally set in place. Six of the 24 sprigs lived for awhile, but only 2 were successful. Sprigs that were anchored with brick and failed did so shortly after they were planted. Water movement probably eroded away enough sediment to allow the buoyant sprigs to float from under the brick.

TREATMENT OF TRANSPLANTS IN PHASE II

The effect of NAPH on marine grasses is apparently similar to its effect on terrestrial plants, primarily inducing rapid and heavy rooting. Ten of the 11 sprigs producing new rhizomic growth were treated with it (Table 2). Because of the small number of transplants attempted and successes achieved, we cannot definitely establish the significance of NAPH in such experiments. Our results indicate to us, however, that NAPH was one of the main factors contributing to transplant success.

Particular care was taken to avoid damaging rhizomes and rhizome apexes of sprigs before and during transplanting. No apparent advantage was gained from this care; invariably old rhizomes withered away and were replaced by new ones developing from the bases of the short-shoots.

MORTALITY OF TRANSPLANTS

Visual checks made throughout the year showed that the most critical period for the survival of turtle grass was during the first 3 months after transplanting. In phase I, mortality of plugs planted in the canal was 60% through the third month (October), 22.5% through the sixth month (January), zero

through the 3-month period February-April, and 2.5% during the remainder of the study. Losses in the control area for the same time intervals were 25, 10, 5, and 5%, respectively.

Mortality experienced during phase II was also high. Over half (57%) of the sprigs transplanted in April failed before the end of the third month (July) and 7% from August to October. Additional failures within this phase might have occurred had the experiment continued through the winter.

CONCLUSIONS AND RECOMMENDATIONS

Our experiments resulted in the first successful field transplantation of turtle grass. All new short-shoots produced by transplants were from the new rhizome apexes (Figure 5). This finding supports the observations of Phillips (1960) and Tomlinson and Vargo (1966) that buds on the rhizome apex are the only source of short-shoots. It is also in agreement with findings in the tank culture of *Thalassia* (Fuss and Kelly, 1969). Continuous growth of turtle grass depends on the activity of vigorous rhizome apexes, but the apexes do not contain the only meristematic tissue in the plant. New rhizomes can be produced from residual meristematic tissue present in the old short-shoot. Phillips (1960) observed such branching in the field and stated that it could account for the continued growth of turtle grass if the apex of the rhizome were damaged or lost, but believed that the frequency of this branching was small. Tomlinson and Vargo (1966) also reported that vegetative branching in short-shoots occurs and indicated that it is rare.

Undamaged leaves may not be required for sprig transplanting. Further studies are needed to determine, for example, if the leaves could be cut back to reduce the surface area and buoyancy of the sprig. Results of investigations in Boca Ciega Bay by Prest, Saloman, and Taylor[7] show that turtle grass leaves clipped as much as 50% of their original height (about 26 cm) would regrow as much as 3 to 4 cm (1.2 to 1.6 inches) per week. It would thus appear that physical damage to leaves is quickly overcome by regrowth of the plant.

We have shown that turtle grass can be transplanted in the field and that it will grow in an area denuded by coastal dredging. A simple transplant method using only the short-shoots of this grass, the hormone NAPH, and construction rod was 100% successful (six transplants) in a land-fill finger canal (Table 2). This method has value for use in restoring *Thalassia* to estuarine environments when conditions favorable for plant growth exist or can be artificially created. We must emphasize however, that no large-scale transplant program has been attempted. Moreover, recent observations (November 1970)[8] of vegetative growth into our original control site indicate that turtle grass spreads at an annual rate of only 20 cm (8 inches) or less.

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EFFECT OF DIETARY FISH OIL ON THE FATTY ACID COMPOSITION AND PALATABILITY OF PIG TISSUES[1]

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ABSTRACT

Basically, this report deals with the problem of a "fishy" flavor in the meat of pigs, which sometimes results when pigs are fed fishery products, such as fish meal, above a certain concentration in the diet.

In this study, pigs were fed diets containing fish oil to investigate specifically: (1) the effect, on the taste of the meat, of feeding pigs fish oil, (2) the effect, on the taste of the meat, of withdrawing the oil from the diet at given times, (3) the fatty acid composition of the various body tissues of the pigs, and (4) the relation of composition to the taste of the meat.

The principal findings of the study were: (1) The amount of the fish oil $\omega 3$ fatty acids fed and deposited was significantly positively correlated with the weighted organoleptic score[4] when the pigs were fed the oil containing diets to a market weight of 90.9 kg. (2) Removal of the fish oil from the pigs' diets when the pigs obtained body weight (of either 68.0 or 79.5 kg) resulted in a loss of the significant positive correlation above. (3) Differences in the degree of unsaturation and in fatty acid composition were found among the oils in the tissues examined. (4) A signifiant positive correlation was obtained between the quantity of the characteristic fatty acids ($\omega 3$) of fish oil fed and the quantity deposited in three of the four tissues examined, the exception being the *longissimus dorsi* tissue.

Both the processors of fishery industrial products and the feed manufacturers who use the products are sometimes confronted with the problem of a fishy flavor in the carcasses of animals fed diets in which these products are included. Fish oil fed directly to the animals or fed as a residual component of fish meal or of fish solubles has been shown to produce an off-flavor under certain conditions (Banks and Hilditch, 1932; Hilditch and Williams, 1964).

Through practical research, the problem has been partly solved by reducing the quantity (that is, the percentage) of fish oil in the diet or by eliminating the oil during an interval of time before the animals are marketed (Frazer, Stothart, and Gutteridge, 1934). This latter technique is not always effective, especially when fairly high (8.25%) levels of fish oil have been fed (Anglemier and Oldfield, 1957).

Investigations to relate more specifically the causal agents of the off flavor resulting from the use of fish oil have led to the hypothesis that the long-chain polyunsaturated fatty acids of the C_{20-22} series commonly found in fish oil are precursors of the flavor-producing components (Banks and Hilditch, 1932; Marion and Woodroof, 1963; Miller, Gruger, Leong, and Knobl, 1967). Investigations by the Animal Nutrition Unit of the Bureau of Commercial Fisheries (now the National Marine Fisheries Service) Technological Laboratory, College Park, Md., using chickens, have indicated that a further partitioning of the C_{20-22} fatty acid series results in a positive correlation between individual fatty acids of these series deposited and the detection of the off-flavor (Miller et al., 1967).

In a continuation of this line of investigation, the work reported here was divided into four experiments. Their purposes were to determine the following information:

1. The relation between the menhaden-oil fatty acid fed and the fatty acid pattern of tissue samples (namely, those of the outer and the in-

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[4] Note the organoleptic score increased with greater unacceptability.

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ner backfat, the *longissimus dorsi* muscle, and the liver) of pigs fed various diets with and without menhaden oil and for various intervals of time before they are marketed.

2. The organoleptic effect of the different dietary levels of menhaden oil on the meat of the pigs and the retention or disappearance of the off-flavor by removal of menhaden oil from the diet of the pigs when they reach a body weight of 68.0 or 79.5 kg and are subsequently marketed when they reach a weight of 90.9 kg.

3. The relation, if any, between the detection of off-flavor and the pattern of fatty acid deposition in the tissue samples.

4. The metabolic interrelation of fatty acids of the various fatty acids of the omega families ($\omega 3$, $\omega 6$, $\omega 9$).

RELATION BETWEEN MENHADEN OIL FATTY ACIDS FED TO PIGS AND DEPOSITIONAL PATTERNS OF THESE FATTY ACIDS IN THE PIG TISSUES

Callow (1935, 1938) indicated that the rate of deposition of fat in pigs is correlated with the iodine number of the fat and that slower growing pigs deposit a more unsaturated fat. Accordingly, we felt that our experimental pigs should be handled so that they would develop uniformly, thus minimizing the variation in the composition of depot fat resulting from differential rates of growth.

The first part of this experiment was a general study to monitor the uniformity of growth of the pigs and of the development of their carcasses. That is, we wanted to determine whether the diets fed and our treatment of the pigs would result in any abnormalities that might invalidate the specific findings in this first experiment and in the other three experiments to follow.

UNIFORMITY OF GROWTH OF PIGS AND OF DEVELOPMENT OF CARCASSES

Uniformity of Growth

Described here are the diets, the allotment and management of the pigs, and the statistical analyses used.

The diets were balanced on an equal-protein and equal-calorie basis and were fortified to supply all the known nutrients required by pigs. Crude menhaden fish oil that had been stabilized with butylated hydroxy toluene[5] was added at levels of 0.4% to 1.4%. The oil replaced various proportions of cerelose and Solka Flox[6] to give isocaloric and isonitrogenous diets (Table 1). The diets were mixed in a ribbon-type mixer and were pelleted weekly through a 12-mm die. Steam was not used in the pelleting process. Table 2 shows the gas-liquid chromatographic analyses of the oil and of the diets fed.

[5] Level of addition is trade secret.
[6] Trade names are used merely to simplify descriptions; no endorsement is implied.

TABLE 1.—Diet formulation used in experiment to determine the dietary level of menhaden oil that will impart off-flavors to the meat of pigs.

Ingredients	Concentration of the given ingredients in the diet when the percentage of menhaden oil in the diet was						
	0	0.4	0.6	0.8	1.0	1.2	1.4
	%	%	%	%	%	%	%
Fixed basal ingredients:							
Corn US #2	67.0	67.0	67.0	67.0	67.0	67.0	67.0
Soybean oil meal	20.3	20.3	20.3	20.3	20.3	20.3	20.3
Alfalfa leaf meal	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Dicalcium phosphate	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Salt (trace mineral)[1]	.6	.6	.6	.6	.6	.6	.6
Vitamin mix[1]	2	2	2	2	.2	2	.2
Variable ingredients:							
Cerelose	6.9	5.5	4.8	4.1	3.4	2.7	2.0
Cellulose	--	1.0	1.5	2.0	2.5	3.0	3.5
Menhaden oil	--	.4	.6	.8	1.0	1.2	1.4

[1] Sufficient trace minerals and vitamins were present to meet the requirements of the National Research Council.

TABLE 2.—Gas chromatographic analysis of methyl esters of the fatty acid components of the menhaden oil and of the diets fed to pigs.

Fatty acid	Concentration of the given fatty acid in menhaden oil	Concentration of the given fatty acid in the diet when the percentage of menhaden oil in the diet was:						
		0	0.4	0.6	0.8	1.0	1.2	1.4
	%	%	%	%	%	%	%	%
[1]14:0	5.96	0.17	1.03	1.42	1.62	1.98	2.05	2.51
14:1	--	0.05	0.05	0.06	0.06	0.08	0.08	0.10
15:0	0.34	0.13	0.05	0.15	0.21	0.06	0.06	0.07
?	[3]tr	0.06	0.17	0.05	0.06	0.27	0.27	0.31
15:1	0.09	--	tr	tr	tr	tr	tr	tr
16:0	13.10	11.77	13.15	13.59	13.26	14.10	13.46	14.85
[2]16:1 ω7	10.36	0.31	1.31	1.59	1.86	2.31	2.34	2.85
17:0	0.64	0.16	0.21	0.24	0.26	0.26	0.27	0.31
?	--	0.11	tr	tr	tr	tr	tr	tr
16:2	--	--	--	--	--	--	--	--
17:1	1.06	0.10	0.23	0.29	0.32	0.36	0.39	0.45
?	0.10	--	0.04	0.04	--	0.03	0.05	0.05
18:0	4.36	2.70	2.86	2.95	3.17	3.16	3.26	3.17
18:1 ω9	27.59	27.45	24.97	24.06	23.57	22.90	22.63	20.44
19:0	1.45	0.10	0.27	0.35	0.42	0.43	0.48	0.58
18:2 ω6	1.57	51.22	44.60	41.86	40.96	39.14	38.22	35.22
?	0.32	tr	tr	tr	tr	tr	tr	tr
?	0.24	0.11	0.13	0.14	0.18	0.12	0.16	0.17
20:0	0.31	0.78	0.78	0.75	0.81	0.65	0.68	0.69
18:3 ω3	0.94	3.31	2.60	2.89	2.75	2.62	2.49	2.97
20:1	1.32	0.40	0.68	0.73	0.76	0.75	0.79	0.86
18:4 ω3	2.89	--	0.50	0.65	0.76	0.84	0.94	1.11
?	0.37	0.07	0.17	0.19	0.24	0.21	0.27	0.30
20:2 ω9	0.18	0.07	0.11	0.12	0.13	0.07	0.13	0.14
20:2 ω6	0.06	tr	tr	tr	tr	tr	tr	0.06
20:3 ω9	0.13	tr	0.08	0.08	0.09	0.05	0.10	0.11
22:3 ω6	0.05	tr	tr	0.04	tr	tr	tr	tr
20:4 ω6	0.69	0.22	0.37	0.42	0.42	0.37	0.45	0.53
22:1 ω2	0.30	tr	0.12	0.09	0.12	0.07	0.13	0.16
20:4 ω3	1.25	0.08	0.29	0.34	0.41	0.42	0.52	0.59
20:5 ω3	12.95	0.11	2.19	2.95	3.35	3.96	4.28	5.01
?	--	0.23	0.40	0.26	0.34	0.15	0.28	0.40
24:0	0.09	--	tr	tr	tr	tr	tr	tr
22:4 ω6	0.59	0.29	0.53	0.47	0.48	0.46	0.54	0.57
22:5 ω6	0.43	--	tr	0.24	tr	0.26	0.46	0.45
22:5 ω3	1.63	--	0.40	0.50	0.63	0.65	0.77	0.86
22:6 ω3	8.47	--	1.71	2.49	2.76	3.29	3.44	4.14

[1] "14:0" means that the fatty acid has 14 carbon atoms per molecule and no unsaturated bond.
[2] "16:1 ω7" means that in the fatty acid the unsaturated bond occurs at the seventh bond from the terminal methyl group.
[3] "tr" means trace.

Seven Yorkshire gilts each weighing about 27.3 kg were allotted to each of the seven treatment groups. Two of the seven pigs of each menhaden-oil group were fed the appropriate oil-containing diet until they attained a body weight of 68.0 kg and then were fed the control diet until they attained a body weight of 90.9 kg. Similarly, two additional pigs of each menhaden-oil group were fed the appropriate oil-containing diet to a body weight of 79.5 kg and then also were fed the control diet to a body weight of 90.9 kg. The remaining three pigs were continuously fed the various test diets containing menhaden oil until they each also attained a body weight of 90.9 kg. Feed was offered twice a day (for a maximum of 1 hr per feeding) to the pigs in individual crate-type pens. This interval of time was considered to be adequate to permit the pigs to eat the same total amount of food that they would have eaten ad lib. Data on rates of gain and consumption of feed were recorded weekly.

Data obtained on rates of gain and utilization of feed were subjected to an analysis of variance (Snedecor, 1956).

Table 3 presents the rates of gain, utilization of feed, and quantity of oil consumed by the pigs fed diets containing the various percentages of menhaden oil.

Results of the analyses of variance for each

TABLE 3.—Rates of gain, utilization of feed, and quantity of oil consumed by pigs fed diets containing various percentages of menhaden oil.

Relative amount of menhaden oil in diet	Average daily gain		Ratio of feed to gain		Mean quantity of oil consumed by pigs to a body weight of		
	Mean	SD	Mean	SD	68.0 kg	79.5 kg	90.9 kg
%	kg	kg			kg	kg	kg
0	0.63	0.065	3.45	0.136	0	0	0
0.4	.64	.065	3.26	.105	0.52	0.62	0.85
0.6	.60	.047	3.34	.093	0.85	0.96	1.30
0.8	.64	.045	3.47	.095	1.03	1.32	1.90
1.0	.64	.025	3.26	.080	1.20	1.48	2.16
1.2	.66	.068	3.28	.100	1.53	1.78	2.70
1.4	.64	.044	3.25	.084	1.61	2.12	3.22

criterion of evaluation indicate that these criteria did not differ significantly.

Development of Carcasses

The yield of lean cuts was obtained as an accumulative value for the four commercial lean cuts—namely, hams, loins, shoulders (picnics), and Boston butts. Cross-sectional measurements of the *longissimus dorsi* muscle of the loin were obtained by cutting the loin at the 10th rib, tracing the muscle area onto paper, and measuring the perimeter of the area by means of a planimeter to convert the encompassed area to square centimeters. The thickness of the backfat was based on an average of three measurements taken at positions opposite the first rib, the last rib, and the last lumbar vertebra.

Table 4 presents the data on the dressing percentage, lean-cut percentage, *longissimus dorsi* area, and backfat thickness obtained from pigs fed the various diets containing menhaden oil. The analyses of variance for each criterion of evaluation indicate that no significant differences occurred among these factors that reveal the growth reaction of the pigs to their diet.

Thus the pigs developed uniformly during the feeding trials. Consequently any differences that may be found in the fatty acid composition of the tissues should be related to the oil in the diet rather than to markedly different growth of the pigs.

RELATION OF DEPOSITIONAL PATTERNS TO FATTY ACIDS IN OIL FED TO PIGS

In this section, we are concerned with the following three subjects: (1) the differences found in the degree of saturation both within and among tissues, (2) the fatty acids identified, and (3) the relations of the quantity of fatty acids fed to the quantity deposited in the various tissues.

Differences Found in Degree of Saturation Both Within and Among Tissues

Described here are (1) the tissue samples used, (2) the extraction of lipids, (3) the preparation of methyl esters, and (4) the quantitative gas-liquid-chromatographic technique.

Samples were taken from the outer and the inner backfat tissue, the *longissimus dorsi*, and the liver in the following manner. From each animal, a sample of backfat was obtained dorsally to the 10th to 12th ribs. This sample was then divided into the "outer" and "inner" fat layers. Samples of the muscle were taken from the eye of the *longissimus dorsi* at the 10th rib. Samples of the liver were taken from the right central lobe. All samples were placed in vials, protected with nitrogen, and held at —20° C until the lipids were extracted from them.

The lipids were extracted from the samples by the homogenization of the tissue in a mechanical blender with a 2:1 mixture of chloroform and methanol for 2 min. The solvent mixture was added in the proportion of 5 ml of mixture to 1 g of sample. The slurry was filtered through a Buchner funnel, and the filter paper and the nonfilterable portion were re-extracted for another 2-min period. The filtrate was evaporated in a rotary vacuum evaporator over a 60° C water bath. The dried sample was redissolved in

TABLE 4.—Dressing percentage, lean-cut percentage, *longissimus dorsi* area, and backfat thickness obtained from pigs fed various diets containing menhaden oil.

Relative amount of menhaden oil in diet	Relative yield of:				*Longissimus dorsi* area		Backfat thickness	
	Dressing		Lean cuts[1]					
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
%	%	%	%	%	cm^2	cm^2	*cm*	*cm*
0	83.8	±2.36	38.9	±2.45	32.39	±5.78	3.56	±0.62
0.4	83.4	±1.23	40.2	±1.68	33.68	±4.83	3.30	± .45
0.6	82.0	±1.89	39.6	±1.07	31.87	±3.68	3.61	± .19
0.8	83.1	±1.06	39.4	±1.32	30.78	±5.08	3.61	± .29
1.0	82.7	±1.62	40.1	±0.88	32.78	±4.39	3.30	± .27
1.2	82.0	±2.21	38.3	±2.14	31.74	±4.00	3.53	± .62
1.4	82.1	±1.86	38.1	±1.61	30.91	±5.19	3.65	± .40

[1] Calculated as the sum of weights of Boston butts, shoulder, loin and ham, as a percentage of weight of dressed carcass.

petroleum ether (30° to 60° C boiling point), poured into a separatory funnel, and washed twice with a 20% solution of NaCl. The layer of petroleum ether was evaporated in the rotary evaporator, and the extracted fat was transferred to containers in which it was protected by nitrogen and was stored at —20° C until methyl esters were prepared from it for analysis.

The methyl esters of the fatty acids were prepared as follows:

Five ml of anhydrous methanol and about 50 mg of freshly cut and shiny sodium were placed into a small test tube. After the sodium had reacted, six to eight drops of the extracted oil were added and heated to reflux on a steam bath for 2 min with agitation. The end point of the reaction was signaled when the solution became clear.

The reaction solution was quenched with 5 ml of distilled water and was transferred to a separatory funnel. The mixture was extracted with two 10-ml portions of petroleum ether (30° to 60° C boiling point). The final water layer was discarded, and the two petroleum ether extracts were combined. The petroleum ether solution was washed with 10 ml of 5% aqueous HCl solution. The acid wash was followed by successive washes with 15-ml and 10-ml aliquots of 20% NaCl solution. The washing was completed when pH paper tested neutral.

The ethereal solution of methyl esters was dried over 3 g of anhydrous Na_2SO_4, filtered, and evaporated over a 60° C water bath, using a vacuum rotary evaporator.

To check for purity, we made a thin-layer chromatogram of the ester solution using silicic acid paper. Methyl myristate was used as the control. A solution of 90 parts petroleum ether, 10 parts ethyl ether, and 1 part formic acid was used to elute the esters. The chromatogram was developed in iodine vapor.

Methyl esters of pure fatty acids were used as reference standards for the C_{14-24} saturated acids, C_{16-24} monoenoic acids, plus linoleic, linolenic, arachidonic, eicosapentaenoic, and docosahexaenoic acids. Also concentrates of 16:2, 16:3, 16:4, and 18:4 methyl esters that were obtained by fractional distillation and urea-inclusion compound fractionalization were used as reference standards.[7] As a secondary reference mixture, methyl esters from whole menhaden oil were also analyzed. From a plot of the logarithms of the retention times (relative to stearate) versus the number of carbon atoms, nearly linear relations were observed for homologous series (Farquhar, Insull, Rosen, Stoffel, and Ahrens, 1959). Identifications were further verified by applying the graphical method of James (1960) for analyses on columns packed with diethylene glycol succinate polyester and Apiezon L. These plots provided the necessary reference data for identification of the various tissue lipids analyzed.[8]

[7] The staff of the National Marine Fisheries Service Technological Laboratory, Seattle, Wash., made the fractional distillations and urea-inclusion compound fractionations.

[8] The fatty acids of the oil fed and of the animal tissues were identified initially in collaboration with the staff of the National Marine Fisheries Service Technological Laboratory, Seattle, Wash.

Methyl esters of fatty acids taken from the various tissues were analyzed with an F&M Biomedical Model 400 gas chromatograph. The instrument was equipped with a hydrogen flame detector. The column used was composed of 4.0-mm ID by 243.8-cm Pyrex glass containing 5.0% (by weight) of diethylene glycol succinate polyester (DEGS from Wilkens Instrument and Research Inc.) supported on 80- to 90-mesh acid-base washed and siliconized flux-calcinated diatomaceous earth (Anakron ABS). The operating conditions were as follows: column temperature, 165° C; flash-heater temperature, 285° C; detector temperature, 200° C; and initial attenuation that corresponds to 10 to 14 amp full-scale deflection. The inlet pressure of the column measured 40 psi of helium, the flow measured 53 ml per min at the outlet of the column. The size of the injected sample was about 0.12 μliter.

The area-percent method was used to determine the corresponding peak areas of the curves obtained from the gas-liquid chromatographic recorder. The fatty acid composition (in average percentage) of each sample was calculated by multiplying peak height by retention time and then multiplying this product by 100 and dividing by the total area.

Certain differences were obtained in the total degree of saturation and quantity of specific

TABLE 5.—Summary of gas-liquid chromatographic analyses indicating comparative degree of unsaturation and quantity of selected fatty acids within and among the tissues obtained from pigs fed either 0% or 1.4% dietary menhaden oil.

Type of fatty acid	Concentration of the various fatty acids in the various tissues when the relative amount of menhaden oil in the diets was:							
	0%				1.4%			
	Backfat				Backfat			
	Inner tissue	Outer tissue	*Longissimus dorsi* tissue	Liver tissue	Inner tissue	Outer tissue	*Longissimus dorsi* tissue	Liver tissue
	%	%	%	%	%	%	%	%
Saturated fatty acids	34.56	28.37	33.74	34.63	35.42	28.77	33.05	34.66
Unsaturated fatty acids	65.44	71.63	62.82	65.37	64.58	71.23	64.62	65.34
Unsaturated bond in the fatty acids:								
1	47.68	49.03	39.38	17.53	47.17	46.16	45.96	15.44
2	16.35	20.49	14.40	16.85	17.39	20.75	11.46	17.43
3	0.93	1.40	1.37	2.35	1.40	1.73	1.15	1.85
4	0.40	0.72	4.95	19.08	0.84	0.88	3.27	12.80
5	0.19	0.27	2.03	2.96	0.80	1.53	2.17	8.52
6	0.03	0.05	0.69	2.26	0.40	0.50	0.62	8.11
Equivalent degree of unsaturation in the fatty acids:								
1	47.68	49.03	39.38	17.53	47.17	46.16	45.96	15.44
2	32.70	40.98	28.80	33.70	34.78	41.50	22.92	34.86
3	2.79	4.20	4.11	7.05	4.20	5.19	3.45	5.55
4	1.60	2.88	19.80	76.32	3.36	3.52	13.08	51.20
5	0.95	1.35	10.15	14.80	4.00	7.65	10.85	42.60
6	0.18	0.30	4.14	13.56	2.40	3.00	3.75	48.66
Total	85.90	98.74	106.38	162.96	95.91	107.02	99.98	198.31
Individually selected fatty acids:								
16:0	19.90	18.49	21.78	13.04	20.39	18.64	19.62	12.80
18:0	12.18	7.78	9.24	19.37	12.49	8.08	10.74	18.76
18:1 ω9	43.67	44.94	34.33	15.86	40.52	42.04	41.16	13.57
18:2 ω6	15.07	18.99	13.24	15.27	16.18	19.40	10.47	15.87
18:3 ω3	0.74	1.18	0.59	0.44	1.02	1.37	0.52	0.57
18:4 ω6	0.34	0.49	2.44	18.29	0.32	0.41	1.61	12.15
20:4 ω3	0.06	0.08	2.42	0.49	0.20	0.31	1.61	0.54
20:5 ω3	0.09	0.13	0.98	0.51	0.27	0.52	1.12	4.18
22:5 ω3	0.10	0.14	1.05	2.45	0.53	1.01	1.04	4.34
22:6 ω3	0.03	0.05	0.69	2.26	0.40	0.50	0.62	8.11

Note: The equivalent degree of unsaturation in the fatty acids was obtained by multiplying the number of double bonds by the quantity of fatty acid.

fatty acids found within and among the tissues examined. All the fatty acids that were identified will be discussed in the next section. For illustrative purposes, Table 5 presents selected results obtained with the various tissues. The outer backfat had the lowest total concentration of saturated fatty acids of all the tissues, regardless of whether the diet contained menhaden oil or did not contain it. The remaining tissues (inner backfat, liver, and *longissimus dorsi*) were all higher than the outer backfat and did not differ markedly from each other in the total concentration of saturated fatty acids.

The difference in degree of saturation when confined to comparisons between the inner and outer backfat is in agreement with reports by Banks and Hilditch (1932) and Sink, Watkins, Ziegler, and Miller (1964). The simple ratio of the total quantity of saturated to unsaturated fatty acids, however, does not describe the true character of the unsaturated fatty acids found within the tissues or among them.

An examination of the quantity of unsaturation on the basis of the number of double bonds and the relative quantities of the corresponding fatty acid groups indicates marked differences among the tissues.

Both the *longissimus dorsi* tissue and the liver tissue contain markedly less fatty acids with one unsaturated bond than do either of the backfat tissues, regardless of the dietary treatment. This difference no doubt is reflected by the 18:1 ω9 content.

The concentration of fatty acids with two unsaturated bonds in the outer backfat tissue is higher than that in the remaining tissues and apparently indicates a differential concentration of 18:2 ω6.

The difference most evident among the tissues with respect to the fatty acids with three unsaturated bonds is the higher concentration found in the liver tissue.

Both the *longissimus dorsi* and the liver tissue contained considerably more of the four-unsaturated-bond fatty acids than did the backfat tissues. The liver, in turn, contained about four times the concentration found in the *longissimus dorsi*. Incorporating menhaden oil into the diet lowered the magnitude of these differences among the tissues.

The relative differences among the tissues in the case of the *longissimus dorsi* tissue reflect about equal quantities of the isomeric fatty acids 20:4 ω6 and 20:4 ω3. The concentration of the fatty acids with four unsaturated bonds in the liver tissue is due primarily to the 20:4 ω6 isomer; only small concentrations of the 20:4 ω3 isomer were found.

Similarly, the concentration of fatty acids with five and six unsaturated bonds in the *longissimus dorsi* and liver tissues was markedly higher than in the backfat tissues. The incorporation of menhaden oil into the diet resulted in increased concentrations of these fatty acids in all tissues, although the differences among tissues were of the same magnitude as the differences occurring in the absence of the menhaden oil. The variable concentrations of the fatty acids with five and six unsaturated bonds, owing to treatment differences, reflect differences in the quantities of 20:5 ω3, 22:5 ω3, and 22:6 ω3 fatty acids.

On the basis of the equivalent degree of unsaturation obtained by the multiplication of the number of unsaturated bonds by the quantity of fatty acids of that category, the relative degree of unsaturation of the four tissues is: inner backfat, 85.9; outer backfat, 98.7; *longissimus dorsi*, 106.4; and liver, 164.0. The incorporation of menhaden oil did not change the relative differences among tissues, but it did result in a treatment difference. The relative degree of unsaturation among the treatments was of the magnitude of 10 to 30 units greater for all tissues except the *longissimus dorsi*.

Thus, these results generally conform with those previously reported that various tissues differ in fatty acid composition (Brown and Deck, 1930; Banks and Hilditch, 1932; Sink et al., 1964) and that dietary oils alter this fatty acid pattern and degree of unsaturation of the animal tissues of monogastric animals (Ellis and Isbell, 1926a, 1926b; Ellis and Zeller, 1930; Ellis, Rothwell, and Pool, 1931; Bhattacharya and Hilditch, 1931; Hilditch and Pedelty, 1940).

Fatty Acids Identified

Table 6 reports the fatty acids identified by the method of gas-liquid chromatographic analysis described in the preceding section.

TABLE 6.—Fatty acids identified in pig tissues.

Fatty acid	Presence or absence of the fatty acid in:			
	Backfat		*Longissimus dorsi* tissue	Liver tissue
	Inner tissue	Outer tissue		
22 6 ω3	+	+	+	+
22 5 ω3	+	+	+	+
20 5 ω3	+	+	+	+
20 4 ω3	+	+	+	+
18 4 ω3	+	+	+	+
18 3 ω3	+	+	+	+
22 5 ω6	–	trace	trace	+
22 4 ω6	–	+	+	+
20 4 ω6	+	+	+	+
20 2 ω6	–	–	+	+
18 2 ω6	+	+	+	+
21:1 ω9	–	–	+	+
20 2 ω9	+	+	+	+
20 1 ω9	+	+	+	+
18 1 ω9	+	+	+	+
22 1 (?)	–	–	trace	+
20 3 (?)	+	+	+	+
16 2	+	+	+	+
16 1 ω7	+	+	+	+
15:1	+	+	trace	+
14 1	+	+	+	+
20 0	+	+	+	+
19 0	+	–	+	+
18 0	+	+	+	+
17 0	+	+	+	+
16 0	+	+	+	+
15 0	+	+	+	+
14 0	+	+	+	+

Twenty-eight fatty acids were identified in the liver tissue, whereas a lesser number was identified in the three other tissues (inner and outer backfat and *longissimus dorsi*). The fatty acids identified included those reported by Sink et al. (1964) plus unsaturated 18, 20, 22 carbon fatty acids of three of the fatty acid families —ω3, ω6, and ω9—according to current classification (Mohrhauer and Holman, 1963a).

With respect to the two backfat tissues, the acids found in addition to those reported by Sink et al. (1964) are as follows: 15:1, 16:2, 20:1 ω9, 18:4 ω3, 20:2 ω9, 20:3, 20:4 ω3, 20:5 ω3, 22:4 ω6, and 22:5 ω3. The liver and *longissimus dorsi* tissue also contained 20:2 ω6, 21:1 ω9, 22:1, 22:5 ω6, and 22:6 ω3. Hill (1966) reported, however, the presence of most of these fatty acids in various tissues of miniature pigs with the exception of 20:4 ω3, which we found in our pigs. All of these fatty acids, except 20:4 ω3, have also been noted in rat tissue (Mohrhauer and Holman, 1963a, 1963b, 1963c), and all of them including 20:4 ω3, have also been noted in chick tissue (Miller et al., 1967), in fish tissues and in seal tissue (Ackman, Burgher, and Jangaard, 1963; Ackman, Jangaard, Hoyle, and Brockerhoff, 1964).

The relation of the fatty acids fed (X) to those deposited in the various tissues (Y) was established by correlation and polynomial regression analyses. A polynomial regression computer program prepared by the Biomedical Division of the University of California, Los Angeles, was used. The extent of analysis of the data was limited to the fourth polynomial degree. Regression coefficients, standard errors of regression, correlation coefficients, analyses of variance, and data plots (predicted and observed) were obtained.

Correlation and polynomial regression analyses of the gas-liquid chromatographic data presented in Tables 7 to 10 indicate that the marine-type polyunsaturated fatty acids of the linolenic acid (ω3) family were deposited in all four tissues examined.

In general, a significant positive correlation was obtained between the quantity of the ω3 fatty acids fed and the quantity deposited in the various tissues. This relation was not obtained, however, with the *longissimus dorsi* tissue. The only explanation we have is that the reaction caused by difficulties in the extraction of the fatty acids and their subsequent separation masked any pattern.

Definite relations between the amounts of most of the ω3 fatty acids fed to pigs and the amounts deposited were found in the liver tissues and in the inner backfat tissues and the outer ones.

Specifically, the quantity of two of the menhaden oil fatty acids (22:5 ω3, and 22:6 ω3) found in the liver was positively correlated (0.01%) with the quantity of oil fed to the pigs until they were of market weight (90.9 kg). The correlation for 20:5 ω3 approached significance.

TABLE 7.—Liver tissue: concentration of fatty acids found in liver tissue and correlation to quantity of various fatty acids fed for various time intervals.

Fatty acid	Pig weight group	Concentration of fatty acid in liver tissue when the percentage of menhaden oil in the diet was:							Correlation coefficient	Kind of regression line
		0	0.4	0.6	0.8	1.0	1.2	1.4		
	Kg	— — — — — — — — *Area percent of fatty acid* — — — — — — — —								
22:6 ω3	90.9	2.25	5.70	7.88	7.72	8.40	8.90	7.49	0.69**	Quadratic
	79.5	2.25	4.65	4.98	5.11	6.25	5.82	7.43	0.70**	Linear
	68.0	2.25	4.30	4.70	4.33	5.69	5.54	5.12	0.65*	Linear
22:5 ω3	90.9	2.55	3.66	4.42	4.48	5.12	5.17	4.85	0.78**	Quadratic
	79.5	2.55	2.94	3.59	3.47	4.00	3.73	4.05	0.56*	Linear
	68.0	2.55	2.70	4.64	3.75	3.70	3.93	4.11	0.59*	Linear
20:5 ω3	90.9	0.56	3.55	6.23	6.52	8.11	8.46	8.14	0.89**	Quadratic
	79.5	0.56	1.57	2.07	1.96	3.33	2.30	2.20	0.50	--
	68.0	0.56	1.00	1.25	1.65	2.12	3.49	2.20	0.67*	Linear
20:4 ω3	90.9	0.39	0.71	0.54	0.50	0.56	0.43	0.44	−0.21	--
	79.5	0.39	1.45	0.75	0.50	0.60	0.69	0.29	−0.42	--
	68.0	0.39	0.58	0.11	0.67	0.71	0.54	0.89	0.39	--
18:4 ω3	90.9	0.08	0.11	0.05	0.08	0.09	0.12	0.07	0.00	--
	79.5	0.08	0.26	0.05	0.07	0.04	0.06	0.06	−0.29	--
	68.0	0.08	0.07	0.05	0.08	0.06	0.10	0.09	0.33	--
18:3 ω3	90.9	0.47	0.67	0.59	0.66	0.73	0.94	0.66	0.26	--
	79.5	0.47	0.77	0.89	0.54	0.56	0.51	0.48	−0.39	--
	68.0	0.47	0.52	0.45	0.57	0.50	0.39	0.59	0.12	--
22:5 ω6	90.9	0.24	0.05	0.06	0.05	0.12	0.11	0.03	−0.27	--
	79.5	0.24	0.23	0.10	0.05	0.17	0.16	0.00	−0.60*	Linear
	68.0	0.24	0.17	0.10	0.10	0.09	0.14	0.03	−0.83**	Linear
22:4 ω6	90.9	1.21	0.43	0.28	0.33	0.29	0.33	0.21	−0.65**	Cubic
	79.5	1.21	0.53	0.34	0.45	0.47	0.48	0.40	−0.63*	Cubic
	68.0	1.21	0.74	0.74	0.64	0.58	0.53	0.63	−0.69*	Linear
20:4 ω6	90.9	18.58	12.37	11.53	10.50	8.82	8.84	9.69	−0.70**	Quadratic
	79.5	18.58	15.23	14.48	14.65	15.12	14.99	12.84	−0.19	--
	68.0	18.58	17.93	16.95	15.98	16.06	16.66	13.92	−0.74**	Linear
20:2 ω6	90.9	0.52	0.19	0.18	0.20	0.14	0.17	0.17	−0.52*	Cubic
	79.5	0.52	0.43	0.50	0.46	0.26	0.40	0.36	−0.34	--
	68.0	0.52	0.20	0.40	0.23	0.40	0.48	0.38	0.13	--
18:2 ω6	90.9	15.87	17.03	16.17	16.32	15.95	15.93	15.79	−0.33	--
	79.5	15.87	14.28	15.69	16.00	17.05	15.93	15.40	0.16	--
	68.0	15.87	16.78	16.27	16.10	16.39	14.48	16.41	−0.12	--
21:1 ω9	90.9	0.27	0.31	0.13	0.15	0.15	0.15	0.14	−0.59**	Linear
	79.5	0.27	0.52	0.45	0.30	0.13	0.25	0.20	−0.40	--
	68.0	0.27	0.42	0.22	0.28	0.29	0.27	0.18	−0.47	--
20:2 ω9	90.9	0.82	0.54	0.60	0.54	0.59	0.58	0.54	−0.12	--
	79.5	0.82	1.29	0.78	0.68	0.44	0.63	0.52	−0.48	--
	68.0	0.82	0.42	0.59	0.56	1.25	0.59	1.05	0.52	--
20:1 ω9	90.9	0.28	0.24	0.19	0.30	0.20	0.23	0.17	−0.22	--
	79.5	0.28	0.44	0.41	0.26	0.20	0.21	0.22	−0.38	--
	68.0	0.28	0.22	0.27	0.25	0.22	0.23	0.25	−0.28	--
18:1 ω9	90.9	16.61	15.28	13.16	14.80	13.56	14.91	14.17	−0.09	--
	79.5	16.61	16.49	18.55	16.18	12.67	13.33	13.56	−0.49	--
	68.0	16.61	15.64	15.55	16.09	14.59	9.68	12.99	−0.54	--

* $P < .05$ ** $P < .01$

Polynomial regression analyses of these data indicate that the incorporation pattern of the fatty acids (20:5 ω3, 22:5 ω3, 22:6 ω3) was quadratic, with the rate of deposition being greater at the lower levels in the diet. Removal of menhaden oil from the diet of the pigs at the two body weights, 68.0 or 79.5 kg, did not alter this pattern markedly. The principal changes were a reduction in the relative degree of significant response (0.01% to 0.05%) and an alteration

TABLE 8.—Inner backfat tissue: concentration of fatty acids found in backfat tissue and correlation to quantity of various fatty acids fed for various time intervals.

Fatty acid	Pig weight group	Concentration of fatty acid in inner backfat tissue when the percentage of menhaden oil in the diet was:							Correlation coefficient	Kind of regression line
		0	0.4	0.6	0.8	1.0	1.2	1.4		
	Kg	*Area percent of fatty acid*								
22:6 ω3	90.9	0	0.13	0.28	0.29	0.54	0.49	0.48	0.76**	Linear
	79.5	0	0.19	0.21	0.51	0.37	0.31	0.40	0.56*	Linear
	68.0	0	0.09	0.15	0.20	1.06	0.28	0.31	0.49	--
22:5 ω3	90.9	0.10	0.35	0.48	0.54	0.96	0.73	0.88	0.77**	Linear
	79.5	0.10	0.25	0.46	0.28	0.60	0.23	0.19	−0.01	--
	68.0	0.10	0.18	0.20	0.39	0.24	0.54	0.51	0.71**	Linear
20:5 ω3	90.9	0.07	0.12	0.18	0.29	0.44	0.41	0.39	0.71**	Linear
	79.5	0.07	0.08	0.17	0.20	0.27	0.16	0.22	0.55*	Linear
	68.0	0.07	0.04	0.11	0.12	0.12	0.33	0.19	0.71**	Linear
20:4 ω3	90.9	0.06	0.15	0.17	0.24	0.34	0.26	0.29	0.72**	Linear
	79.5	0.06	0.11	0.14	0.23	0.34	0.19	0.17	0.43	--
	68.0	0.06	0.06	0.10	0.19	0.11	0.14	0.14	0.89**	Linear
18:4 ω3	90.9	0.06	0.10	0.13	0.13	0.18	0.16	0.18	0.70**	Linear
	79.5	0.06	0.10	0.11	0.11	0.17	0.06	0.12	0.15	--
	68.0	0.06	0.06	0.04	0.08	0.10	0.15	0.30	0.54	--
18:3 ω3	90.9	0.74	0.82	0.89	0.94	1.04	1.02	1.10	0.52*	Linear
	79.5	0.74	0.72	0.88	0.92	1.08	0.77	0.82	0.17	--
	68.0	0.74	0.86	0.80	0.77	1.03	0.71	1.15	0.52	--
22:5 ω6	90.9									
	79.5				Not identified				--	--
	68.0									
22:4 ω6	90.9									
	79.5				Not identified				--	--
	68.0									
20:4 ω6	90.9	0.34	0.33	0.33	0.29	0.39	0.44	0.33	0.03	--
	79.5	0.34	0.28	0.35	0.38	0.34	0.32	0.33	0.09	--
	68.0	0.34	0.36	0.30	0.34	0.34	0.32	0.28	−0.60*	Linear
20:2 ω6	90.9									
	79.5				Not identified				--	--
	68.0									
18:2 ω6	90.9	15.07	15.53	15.45	15.25	17.51	16.30	17.38	0.41	--
	79.5	15.07	13.15	15.43	16.46	17.97	15.16	14.06	0.10	--
	68.0	15.07	17.45	16.70	14.69	17.48	13.12	17.09	−0.01	--

* $P < .05$ ** $P < .01$

in the patterns of incorporation of these fatty acids (quadratic to linear).

No statistical relation was found for the remaining three fatty acids (18:3 ω3, 18:4 ω3, and 20:4 ω3).

Results of statistical analyses of the data obtained with the inner backfat tissue and the outer backfat tissue, however, indicate that all six of the fatty acids of the ω3 family are deposited in the tissue in proportion to the quantity consumed by the pigs. When menhaden oil was fed to the pigs until they attained a body weight of 90.9 kg, a highly significant positive correlation was obtained for all six ω3 fatty acids. Removing the oil from the diet of the pigs when they weighed 79.5 kg changed the pattern slightly with respect to the outer backfat tissue. The quantity of 18:4 ω3 fed was no longer correlated with the amount deposited. Removal of the oil when the pigs weighed 68.0 kg resulted in only three of the ω3 fatty acids (20:5 ω3, 22:5 ω3, and 22:6 ω3) being correlated.

Similarly with respect to inner backfat tissue, removal of the menhaden oil when the pigs weighed either 68.0 or 79.5 kg resulted in the quantity of certain of the ω3 fatty acids fed no longer being correlated with the quantity deposited. Polynomial regression analyses of

TABLE 9.—Outer backfat tissue: concentration of fatty acids found in outer backfat tissue and correlation to quantity of various fatty acids fed for various time intervals.

Fatty acid	Pig weight group	Concentration of fatty acid in outer backfat tissue when the percentage of menhaden oil in the diet was:							Correlation coefficient	Kind of regression line
		0	0.4	0.6	0.8	1.0	1.2	1.4		
	Kg	*Area percent of fatty acid*								
22.6 ω3	90.9	0.04	0.23	0.37	0.47	0.59	0.69	0.58	0.85**	Linear
	79.5	0.04	0.25	0.24	0.35	0.63	0.30	0.49	0.58*	Linear
	68.0	0.04	0.20	0.26	0.16	0.34	0.33	0.43	0.74**	Linear
22.5 ω3	90.9	0.14	0.54	0.52	0.85	1.01	1.06	1.14	0.89**	Linear
	79.5	0.14	0.52	0.52	0.66	0.85	0.78	1.04	0.85**	Linear
	68.0	0.14	0.36	0.19	0.63	0.72	0.59	0.85	0.84**	Linear
20.5 ω3	90.9	0.13	0.19	0.24	0.40	0.49	0.56	0.68	0.89**	Linear
	79.5	0.13	0.13	0.21	0.30	0.40	0.28	0.57	0.79**	Linear
	68.0	0.13	0.17	0.20	0.17	0.28	0.24	0.33	0.74**	Linear
20.4 ω3	90.9	0.08	0.14	0.15	0.28	0.33	0.27	0.38	0.76**	Linear
	79.5	0.08	0.10	0.13	0.20	0.23	0.23	0.37	0.84**	Linear
	68.0	0.08	0.10	0.12	0.26	0.20	0.22	0.19	0.41	---
18.4 ω3	90.9	0.13	0.12	0.12	0.17	0.17	0.19	0.20	0.58**	Linear
	79.5	0.13	0.18	0.11	0.14	0.13	0.34	0.19	0.36	--
	68.0	0.13	0.07	0.11	0.09	0.15	0.13	0.10	0.18	--
18.3 ω3	90.9	1.12	1.24	1.23	1.26	1.32	1.29	1.51	0.49*	Linear
	79.5	1.12	1.11	1.31	1.33	1.32	1.29	1.34	0.72**	Linear
	68.0	1.12	1.24	1.25	1.20	1.12	1.18	1.24	0.08	--
22.5 ω6	90.9	0.02	trace quantities						--	--
	79.5								--	--
	68.0								--	--
22.4 ω6	90.9	0.11	0.08	0.05	0.11	0.11	0.04	0.08	--	--
	79.5	0.11	0.09	0.07	0.10	0.06	0.07	--	--	--
	68.0	0.11	0.05	0.13	0	0.11	0.11	0.11	--	--
20.4 ω6	90.9	0.49	0.44	0.36	0.44	0.43	0.35	0.43	−0.26	--
	79.5	0.49	0.43	0.44	0.42	0.41	0.38	0.44	−0.28	--
	68.0	0.49	0.42	0.53	0.19	0.45	0.51	0.34	−0.16	--
20:2 ω6	90.9								--	--
	79.5	Not identified							--	--
	68.0								--	--
18.2 ω6	90.9	18.99	19.80	19.58	19.33	19.90	19.20	20.72	0.04	--
	79.5	18.99	17.28	21.56	20.43	20.39	19.32	19.26	0.13	--
	68.0	18.99	20.31	21.27	19.35	18.60	19.89	18.22	−0.36	--

* $P < .05$ ** $P < .01$

these data indicate that, where a correlation existed, the data relative to the incorporation of the ω3 fatty acids were linear.

EFFECT OF MENHADEN OIL CONSUMPTION ON ORGANOLEPTIC EVALUATION OF PIG TISSUE

This second part of the study was made to determine (1) the organoleptic effect on the meat of the pigs fed different levels of menhaden oil in their diet and (2) the retention or disappearance of off-flavors by removal of menhaden oil from the diet of the pigs when they attained a body weight of 68.0, 79.5, or 90.9 kg.

TRIAL I: LEVEL OF MENHADEN OIL THAT RESULTS IN A FISHY FLAVOR

As was just indicated, Trial I was conducted to determine if menhaden oil fed at various levels in the diet would cause off- (fishy) flavor in pork. The levels used in the experiment bracketed a 1.0% level, which was reported by Vestal, Shrewsbury, Jordon, and Milligan (1945) to cause a fishy flavor.

Experimental Procedure

Reported here are the diets, management, samples, and tests.

TABLE 10.—*Longissimus dorsi* tissue: concentration of fatty acids found in *longissimus dorsi* tissue and correlation to quantity of various fatty acids fed for various time intervals.

Fatty acid	Pig weight group	Concentration of fatty acid in *longissimus dorsi* tissue when the percentage of menhaden oil in the diet was:							Correlation coefficient	Kind of regression line
		0	0.4	0.6	0.8	1.0	1.2	1.4		
	Kg	*Area percent of fatty acid*								
22:6 ω3	90.9	0.23	0.94	0.79	0.78	0.91	0.89	0.51	−0.09	--
	79.5	0.23	0.37	0.59	0.58	0.64	1.26	0.90	0.46	--
	68.0	0.23	0.47	0.47	0.25	0.42	0.48	0.85	0.46	--
22:5 ω3	90.9	0.32	1.15	1.34	0.85	1.02	0.85	0.85	--	--
	79.5	0.32	0.76	0.85	0.94	0.79	1.70	1.25	0.75**	Linear
	68.0	0.32	1.05	0.59	0.47	0.78	0.57	1.14	0.25	--
20:5 ω3	90.9	0.19	1.61	1.68	1.09	1.23	1.24	1.31	0.01	--
	79.5	0.19	0.47	0.98	0.58	0.52	1.88	1.47	0.43	--
	68.0	0.19	0.30	0.69	0.51	0.63	0.44	0.77	0.59*	Linear
20:4 ω3	90.9	0.53	2.42	2.87	0.92	0.91	0.89	0.87	−0.34	--
	79.5	0.53	0.23	1.24	2.30	0.21	1.51	0.28	0.05	--
	68.0	0.53	0.12	1.04	0.94	0.94	2.15	3.03	0.10	--
18:4 ω3	90.9	0.04	0.08	0.09	0.03	--	0.07	0.10	--	--
	79.5	0.04	0.05	--	0.24	--	--	--	--	--
	68.0	0.04	0.04	--	0.10	0.16	0.16	--	--	--
18:3 ω3	90.9	0.45	0.53	0.64	0.56	0.58	0.56	0.57	0.07	--
	79.5	0.45	0.38	0.53	0.62	0.43	0.63	0.44	0.20	--
	68.0	0.45	0.77	1.05	0.81	0.57	0.51	0.50	−0.23	--
22:5 ω6	90.9	0.02	0.06	0.09	0.12	--	--	--	−0.22	--
	79.5	0.02	tr	tr	0.18	tr	tr	tr	--	--
	68.0	0.02	tr	tr	0.04	0.29	tr	tr	--	--
22:4 ω6	90.9	0.34	0.41	0.84	0.37	--	0.32	0.16	−0.26	--
	79.5	0.34	0.48	0.40	0.22	0.15	0.19	0.16	0.09	--
	68.0	0.34	0.37	0.27	0.12	0.16	0.17	0.36	0.23	--
20:4 ω6	90.9	2.42	2.85	2.13	2.35	1.92	1.65	1.32	−0.58*	Linear
	79.5	2.42	2.04	3.00	1.62	1.52	3.55	2.64	0.10	--
	68.0	2.42	2.00	2.12	0.74	1.53	0.92	1.39	−0.44	--
20:2 ω6	90.9	0.13	0.19	0.25	0.09	tr	0.05	tr	−0.46*	Linear
	79.5	0.13	0.11	0.10	tr	0.05	tr	tr	−0.68**	Linear
	68.0	0.13	0.10	tr	0.05	0.11	tr	0.09	−0.21	--
18:2 ω6	90.9	9.46	12.54	10.18	10.96	11.08	10.34	9.65	−0.25	--
	79.5	9.46	10.56	13.39	9.45	11.44	17.35	13.84	0.53*	Linear
	68.0	9.46	15.63	10.14	12.36	10.39	8.81	9.61	−0.39	--

* $P < .05$ ** $P < .01$

Table 1 shows how the diets used were formulated, and Table 2 shows the gas-liquid-chromatographic analyses of the menhaden oil and the diets fed. Management and allotment are described in Table 11.

The right loin of each animal was collected, and three slices, each 1.37 cm thick, were cut proceeding posteriorly from the 10th rib. The slices and the remaining portion of the loin were held in frozen storage until used in the taste tests to be described shortly.

The slices of loin were placed in uncovered pans, one-half cup of water was added to each pan, and the pans were held for 50 min in a gas oven at 163.0° C. No seasoning was added to the samples. The remaining portions of the loins, which were used in a home-consumer test described in the next section, were prepared

TABLE 11.—Design of Trial I to determine the level of menhaden oil in the diet that will impart a fishy flavor to the meat of pigs.

Level of menhaden oil in the diet	Pigs allotted per treatment
%	*Number*
0	3
0.4	3
0.6	3
0.8	3
1.0	3
1.2	3
1.4	3

using variable cooking times and temperatures. Salt and pepper were the only condiments used.

Two tests were made: a panel test and a home-consumer test. Twelve panel members tested (once daily) a portion of the loin from various animals in a triangular test pattern. In addition to matching like samples, the panel members indicated a score according to the numerical standard: 1 (good) to 10 (inedible) and made any additional subjective comments that they felt would be helpful concerning the samples. The remaining portions of the loins were distributed randomly to staff members and were accompanied with a form requesting a description of the method of cooking used, a statement of the number of persons tasting, and a subjective evaluation of the flavor.

Results and Discussion

Tables 12, 13, and 14 present the results of Trial I, which was conducted to establish the level of fish oil in the diet that would induce a fishy flavor in pork. The results, as presented in Table 13, are somewhat misleading, because two facts need to be considered in interpreting them. First, part way through the taste test, the freezer in which the test samples were stored malfunctioned. The samples of meat thawed for 2 days and then refroze. Subsequently, a number of panelists detected off-flavors in the control sample as well as in the samples from the pigs receiving the lower levels of fish oil. Second, one panelist continuously detected off-flavor and fishiness regardless of the dietary treatment. In view of these two facts, Table 14 is included; here results are presented of tests conducted before the freezer malfunctioned and without the evaluations of the one panelist. The organoleptic results (Table 14) indicate that an off-flavor in pork could be detected when pigs consumed menhaden oil at a level of 0.8% of their diet and that a fishy flavor could be detected when the pigs consumed menhaden oil at a level of 1.2% of their diet and were fed when they attained a weight of 40.5 kg until they attained a market weight of 90.9 kg.

These results confirm a previous report by Vestal et al. (1945), which established that a level of 1.0% menhaden oil in the diet would cause a fishy flavor.

The results of the home-consumer test agree in general with those of the panel test that a fishy flavor was detected when menhaden oil was fed at a level of 1.2% in the diet. The one indication of off-flavor and fishy flavor in the control sample was found by the judge who had consistently done so in the panel test. All samples in the home-consumer test had been subjected to the thawing and refreezing process.

TABLE 12.—Panel test Trial I—organoleptic results obtained with loins of pigs fed menhaden oil at various levels in the diet.

Concentration of menhaden oil in diet	Samples tested	Detection of adverse flavor	
		Off	Fishy
%	*Number*	*Number*	*Number*
0	18	14	3
0.4	3	7	1
0.6	3	4	1
0.8	3	10	2
1.0	3	5	1
1.2	3	10	5
1.4	3	13	3

TABLE 13.—Panel test Trial I—organoleptic results (selected data) obtained with loins of pigs fed menhaden oil at various levels in the diet.

Concentration of menhaden oil in diet	Sample tested	Detection of adverse flavor	
		Off	Fishy
%	*Number*	*Number*	*Number*
0	11	0	0
0.4	1	0	0
0.6	2	2	0
0.8	2	2	0
1.0	3	4	0
1.2	2	4	2
1.4	1	6	1

TABLE 14.—Home-consumer test Trial I—organoleptic results obtained with loins of pigs fed menhaden oil at various levels in the diet.

Concentration of menhaden oil in diet	Samples tested	Testers	Detection of adverse flavor	
			Off	Fishy
%	*Number*	*Number*	*Number*	*Number*
0	14	48	1	1
0.4	6	28	6	0
0.6	6	12	0	0
0.8	6	18	1	0
1.0	6	25	1	0
1.2	5	9	2	2
1.4	3	22	7	7

The fact that persons unaware of the feeding regimen were less able to detect the off-flavor may indicate that the members of the test panel were overly critical in their evaluation. In view of the subjectivity of organoleptic tests, we felt that the aim of the trial was attained and that a suitable gradient in the concentration of fish oil in the diet was established for the further study of fishy flavor.

TRIAL II: RELATION OF FLAVOR OF MEAT TO BODY WEIGHT AT TIME MENHADEN OIL WAS REMOVED FROM DIET

As in Trial I, in Trial II loin samples were used in two organoleptic tests (panel and home-consumer) to determine if any fishy taste was imparted to animals fed the experimental diets. Twelve panel members tested six loin samples (*longissimus dorsi* and inner backfat) per day during the test. The panelists were asked to pick the control and to score each sample (lean and fat) on a numerical scale of 1 (good) to 5 (inedible). In addition, the members of the panel were asked to describe, subjectively, the flavor of the samples. All panelists were aware of the experimental design.

A home-consumer test was conducted in the same way as in Trial I.

The diets used were formulated and prepared in a manner similar to that indicated in Table 1. Table 2 shows the gas-liquid chromatographic analyses of the oil and of the diets fed.

The samples were collected and prepared as in Trial I.

The results of both organoleptic tests (panel and home-consumer, Tables 15 and 16) agree with those obtained in Trial I. In the panel tests, the results indicate that off-flavors were detected at the 0.8% level of menhaden oil in the diet and that a fishy flavor was detected at the 1.0% level.

In the home-consumer tests, a fishy flavor was not detected until the pigs were fed menhaden oil at the 1.2% level in the diet.

TABLE 15.—Panel test Trial II—organoleptic results obtained with *longissimus dorsi* and inner backfat tissue of pigs fed various levels of menhaden oil in the diet until the pigs attained a body weight of 90.9, 79.5, or 68.0 kg.

Concentration of menhaden oil in diet	Weight of pigs when oil was omitted from diet	Samples tested	Detection of adverse flavor	
			Off	Fishy
%	*Kg*	*Number*	*Number*	*Number*
0	--	7	0	[1]
0.4	90.9	3	1	1
	79.5	2	0	2
	68.0	2	0	0
0.6	90.9	3	0	0
	79.5	3	0	0
	68.0	1	0	0
0.8	90.9	3	0	0
	79.5	2	0	1
	68.0	2	1	2
1.0	90.0	3	0	0
	79.5	2	0	1
	68.0	2	3	6
1.2	90.9	4	0	0
	79.5	2	2	2
	68.0	1	3	3
1.4	90.9	2	0	0
	79.5	2	1	2
	68.0	2	0	0

[1] As was indicated in Trial I, one panelist detected off-flavor and fishy flavor in the control sample and in samples from pigs fed the low levels of menhaden oil in the diet. Because this panelist was unable to distinguish between the control and the test samples, he was replaced.

TABLE 16.—Home-consumer test Trial II—organoleptic results obtained with *longissimus dorsi* and inner backfat tissue of pigs fed various dietary levels of menhaden oil in the diet until the pigs attained a body weight of 90.9, 79.5, and 68.0 kg.

Concentration of menhaden oil in diet	Weight of pigs when oil was omitted from diet	Samples tested	Number of testers	Detection of adverse flavor	
				Off	Fishy
%	*Kg*	*Number*	*Number*	*Number*	*Number*
0	--	7	21	0	0
0.4	90.9	3	15	0	0
	79.5	2	3	0	0
	68.0	2	12	0	0
0.6	90.9	3	8	0	0
	79.5	3	11	0	0
	68.0	1	5	0	0
0.8	90.9	3	8	0	0
	79.5	2	6	0	0
	68.0	2	4	2	0
1.0	90.9	3	10	0	0
	79.5	2	9	5	0
	68.0	2	12	0	0
1.2	90.9	4	19	3	3
	79.5	2	5	0	0
	68.0	1	4	0	0
1.4	90.9	2	4	2	0
	79.5	2	3	3	3
	68.0	2	8	0	0

RELATION OF MENHADEN OIL FATTY ACIDS DEPOSITED TO ORGANOLEPTIC VALUES OBTAINED WITH PIG TISSUES

This third part of the study was made to determine if a relation exists between the degree of off-flavor detection and the fatty acid deposition pattern of the samples of pig tissue.

PROCEDURE

The details of management, patterns of fatty acid deposition in the tissues, and organoleptic tests were the same as those described earlier.

To establish the relation (if any) of the characteristic polyunsaturated $\omega 3$ fatty acids of menhaden oil to the off-flavor of pig tissue, we first had to establish a positive correlation (if any) between the concentrations of these fatty acids fed to the pigs to the concentrations of the fatty acids deposited in the various pig tissues. Once such a correlation (if it existed) was established, then the transformational relation of the concentration of fatty acids fed and deposited to the organoleptic evaluation could be undertaken.

Results of gas-liquid chromatographic analyses of the diets fed (Table 2) indicate that, in general, as the percentage of menhaden oil in the diet increased, the percentage of linolenic $\omega 3$ family acids (18:3 $\omega 3$, 18:4 $\omega 3$, 20:4 $\omega 3$, 22:5 $\omega 3$, and 22:6 $\omega 3$) characteristic of menhaden oil increased proportionately in the diet.

To determine whether the concentrations of these fatty acids in the diet are correlated with the taste of the pig flesh, we had to develop a weighted numerical score of organoleptic data. We obtained the weighted score for each sample tested by multiplying the number of testers times the numerical values of their scores and summing to a total. For example, if five of the panelists scored the sample 3 and if seven scored the sample 4, the weighted score would be $5 \times 3 = 15$ plus $7 \times 4 = 28$, or a total of $15 + 28 = 43$. The weighted scores were used as the Y axis, and the quantity of oil in kilograms or in percent consumed by each pig was used as the X axis in a subsequent correlation and polynomial regression analysis.

Although four tissues were examined with respect to the deposition of $\omega 3$ fatty acid, only two of these tissues (the inner backfat and the *longissimus dorsi*) were evaluated organoleptically. This comparison was further limited in view of the lack of correlation between the amount in the diet of $\omega 3$ fatty acids fed and the concentration of these fatty acids deposited in the *longissimus dorsi* (Tables 17, 18, and 19). Consequently, the relation of the concentration of the $\omega 3$ family fatty acids deposited in the inner backfat and the organoleptic score obtained with this tissue was used for the comparison of the relation of the concentration of the $\omega 3$ family fatty acids to the organoleptic score.

Because all six of the marine polyunsaturated ($\omega 3$) family fatty acids deposited in the inner backfat tissue were positively correlated with

TABLE 17.—Pigs fed to 90.9 kg [correlation and polynomial regression analyses of menhaden oil consumed (X) to individual fatty acids deposited (Y) in *longissimus dorsi* tissue of pigs when oil was fed until the pigs attained a body weight of 90.9 kg].

Fatty acid fed and deposited	Correlation coefficient	Regression coefficient	Standard error of regression	Last degree of polynomial significant	
				Degree	F value
22 6 $\omega 3$	−0.09	−0.016	0.041	--	0.15
22 5 $\omega 3$	--	--	--	--	--
22 5 $\omega 6$	−0.22	−0.038	0.041	--	0.85
22:4 $\omega 6$	−0.26	−0.052	0.048	--	1.19
20 5 $\omega 3$	0.01	0.003	0.077	--	0.00
20:4 $\omega 3$	−0.34	−0.029	0.191	--	2.26
22:1 (?)	--	--	--	--	--
20 4 $\omega 6$	−0.58**	−0.220	0.074	Linear	8.76**
20:3	−0.22	−0.022	0.024	--	0.84
21:1 $\omega 9$	−0.32	−0.029	0.021	--	1.96
20:2 $\omega 6$	−0.46*	−0.036	0.017	Linear	4.56*
20 2 $\omega 9$	−0.34	−0.072	0.048	--	2.24
18 4 $\omega 3$	--	--	--	--	--
20:1 $\omega 9$	0.02	0.002	0.024	--	0.01
18 3 $\omega 3$	0.07	0.004	0.014	--	0.09
20.0	−0.01	−0.001	0.016	--	0.00
18 2 $\omega 6$	−0.25	−0.210	0.194	--	1.15
19:0	0.29	0.047	0.039	--	1.50
18.1 $\omega 9$	0.46*	1.130	0.527	Linear	4.59*
18:0	0.09	--	--	--	--
16 2	−0.04	−0.002	0.013	--	0.02
17:0	−0.05	−0.009	0.045	--	0.04
16.1 $\omega 7$	−0.09	−0.035	0.099	--	0.13
16:0	−0.04	−0.054	0.300	--	0.03
15 1	--	--	--	--	--
15 0	−0.22	−0.026	0.027	--	0.89
14 1	−0.06	−0.009	0.038	--	0.06
14 0	−0.20	−0.033	0.038	--	0.74

* $P < .05$ ** $P < .01$

the concentration of menhaden oil in the diet fed, the quantity of oil consumed (X) could be compared with the organoleptic scores (Y) obtained for the pigs in each weight group (68.0, 79.5, 90.9 kg).

RESULTS

Tables 20, 21, and 22 give the weighted organoleptic scores obtained from the panel organoleptic tests. (Larger numerical values indicate an unacceptable product or a trend toward an unacceptable product.)

Statistical analyses of these data indicate a positive correlation between increased consumption of oil and higher organoleptic scores for tissues from pigs fed the oil until they attained a body weight of 90.9 kg (Table 23). Removal of the oil from the diet of the pigs at a body weight of either 68.0 or 79.5 kg resulted in a

TABLE 18.—Pigs fed to a body weight of 79.5 kg [correlation and polynomial regression analyses of menhaden oil consumed (X) to individual fatty acids deposited (Y) in *longissimus dorsi* tissue of pigs when oil was fed until the pigs attained a body weight of 79.5 kg].

Fatty acid fed and deposited	Correlation coefficient	Regression coefficient	Standard error of regression	Last degree of polynomial significant	
				Degree	F value
22 6 ω3	0 46	0 189	0 104	--	3 29
22 5 ω3	0.75**	0 235	0 059	Linear	15 82**
22 5 ω6	--	--	--	--	--
22 4 ω6	0 09	0 012	0 039	--	0 10
20 5 ω3	0 43	0 170	0 103	--	2 73
20 4 ω3	0 05	0 036	0 224	--	0 03
22 1 (?)	--	--	--	--	--
20 4 ω6	0 10	0 069	0 209	--	0 11
20 3	0 27	0 028	0 029	--	0 91
21 1 ω9	−0 65**	−0 039	0 013	Linear	8 70*
20 2 ω6	−0 68**	−0 039	0 012	Linear	10 47**
20 2 ω9	−0 39	−0 123	0 084	--	2 17
18 4 ω3	--	--	--	--	--
20 1 ω9	−0 45	−0 092	0 053	--	3 05
18 3 ω3	0 20	0 020	0 027	--	0 51
20 0	−0 05	−0 006	0 029	--	0 04
18 2 ω6	0 53*	1 216	0 557	Linear	4 77*
19 0	−0 02	−0 002	0 027	--	0 00
18 1 ω9	−0 36	−1 085	0 815	--	1 77
18 0	−0 17	−0 172	0 290	--	0 35
16 2	0 11	0 014	0 037	--	0 14
17 0	−0 11	−0 017	0 043	--	0 15
16 1 ω7	−0 32	−0 101	0 088	--	1 33
16 0	−0 58*	−0 918	0 372	Linear	6 09*
15 1	--	--	--	--	--
15 0	−0 04	−0 006	0 046	--	0 02
14 1	0 32	0 038	0 032	--	1 35
14 0	−0 26	−0 039	0 043	--	0 84

* $P < 05$ ** $P < 01$

TABLE 19.—Pigs fed to a body weight of 68.0 kg [correlation and polynomial regression on analyses of menhaden oil consumed (X) to individual fatty acids deposited (Y) in *longissimus dorsi* tissue of pigs when oil was fed until the pigs attained a body weight of 68.0 kg].

Fatty acid fed and deposited	Correlation coefficient	Regression coefficient	Standard error of regression	Last degree of polynomial significant	
				Degree	F value
22 6 ω3	0 46	0 113	0 073	--	2 38
22 5 ω3	0 24	0 087	0 120	--	0 53
22 5 ω6	--	--	--	--	--
22 4 ω6	0 23	0 049	0 068	--	0 52
20 5 ω3	0 59*	0 130	0 059	--	4 79
20 4 ω3	0 10	0 201	0 680	--	0 09
22 1 (?)	--	--	--	--	--
20 4 ω6	−0 44	−0 363	0 247	--	2 16
20 3	−0 20	−0 029	0 047	--	0 38
21 1 ω9	0 38	0 039	0 032	--	1 50
20 2 ω6	−0 21	−0 012	0 019	--	0 41
20 2 ω9	−0 43	−0 301	0 212	--	2 02
18 4 ω3	--	--	--	--	--
20 1 ω9	−0 42	−0 139	0 101	--	1 91
18 3 ω3	−0 23	−0 056	0 078	--	0 51
20 0	−0 27	−0 042	0 050	--	0 68
18 2 ω6	−0 39	−1 081	0 845	--	1 64
19 0	−0 24	−0 057	0 077	--	0 55
18 1 ω9	0 44	1 392	0 944	--	2 17
18 0	0 40	0 556	0 422	--	1 73
16 2	−0 13	−0 036	0 094	--	0 14
17 0	−0 12	−0 029	0 077	--	0 14
16 1 ω7	0 08	0 080	0 313	--	0 06
16 0	−0 16	−0 531	1 065	--	0 25
15 1	--	--	--	--	--
15 0	−0 03	−0 013	0 129	--	0 01
14 1	−0 08	−0 019	0 080	--	0 06
14 0	−0 11	−0 029	0 083	--	0 12

* $P < 05$ ** $P < 01$

TABLE 20.—Panel test Trial II—weighted organoleptic scores obtained with inner backfat of pigs fed various levels of menhaden oil in the diet until the pigs attained a body weight of 90.9 kg.

Quantity of oil consumed (X)		Weighted organoleptic score (Y)
Kg	*% of diet*	
0	0	21
0 81		23
83	0 4	21
91		27
1 26		22
1 29	0 6	22
1 36		25
1 77		31
1 86	0 8	26
2 07		32
2 00		30
2 15	1 0	34
2 35		35
2 55		29
2 74	1 2	36
2 74		28
2 82		--
3 20	1 4	37
3 25		32

TABLE 21.—Panel test Trial II—weighted organoleptic scores obtained with inner backfat of pigs fed various levels of menhaden oil in the diet until the pigs attained a body weight of 79.5 kg.

Quantity of oil consumed (X)		Weighted organoleptic score (Y)
Kg	*As % of diet*	
0	0	21
0.55	0.4	25
.64		27
.94	0.6	20
.99		21
.99		18
1.30	0.8	20
1.34		20
1.36	1.0	35
1.48		23
1.77	1.2	34
2.11	1.4	32

TABLE 22.—Panel test Trial II—weighted organoleptic scores obtained with inner backfat of pigs fed various levels of menhaden oil in the diet until the pigs attained a body weight of 68.0 kg.

Quantity of oil consumed (X)		Weighted organoleptic score (Y)
Kg	*As % of diet*	
0	0	21
0.45		23
.54	0.4	20
.72	0.6	21
.98	0.8	23
1.09		22
1.21	1.0	22
1.21		25
1.54	1.2	26
1.61	1.4	23
1.61		17

TABLE 23.—Correlation and polynomial regression analyses of quantity of menhaden oil consumed (X) to weighted organoleptic score (Y) when the oil was fed until the pigs attained a body weight of 90.9, 79.5, or 68.0 kg.

Oil fed to	Correlation coefficient	Regression coefficient	Standard error of regression	Last degree of polynomial significant	
				Degree	F value
Kg					
90.9	0.82**	2.169	0.371	Linear	329.58**
79.5	.49	2.375	1.325	--	3.21
68.0	.21	0.617	0.967	--	0.41

** $P < .01$

loss of the significant positive correlation between the variables, although the correlation coefficient obtained for the group weighing 79.5 kg approached significance.

These results of organoleptic tests are in agreement with reports of Miller et al. (1967), which indicate that $\omega 3$ family fatty acicds, when fed and subsequently deposited, are positively correlated with organoleptic scores obtained with broiler flesh. The results are in partial agreement with the hypothesis of Banks and Hilditch (1932), who suggested that the fatty acids of the C_{20-22} series are associated with an off- (fishy) flavor. Both the results reported here and those reported by Miller et al. (1967) indicate that fatty acids of the $\omega 3$ family containing 18 to 22 carbon atoms are positively correlated with the incidence and degree of off-flavor in pig or broiler flesh. These fatty acids may be causal agents for the off-flavor, or they may not be. In fact, they probably are the precursors of the compound producing the off-flavor.

In these experiments, the inclusion of the menhaden oil in the diet of the pigs resulted in no physiological abnormalities other than the production of off-flavor and an alteration in the pattern of fatty acids in the tissues. This result was not unexpected, because previous work at the National Marine Fisheries Service Technological Laboratory at College Park had indicated that levels of menhaden oil in excess of 10% of the diet are necessary to produce the physiological abnormalities of exudative diathesis and muscular dystrophy experimentally. Adding various antioxidants (vitamin E, selenium, and ethoxyquin) to the diet at compensatory levels prevented the development of these abnormalities (exudative diathesis and muscular dystrophy) in chicks fed menhaden oil at high concentrations (Miller, Leong, Knobl, and Gruger, 1965).

METABOLIC INTERACTIONS OF FATTY ACIDS OF THE OMEGA FAMILY ($\omega 3$, $\omega 6$, $\omega 9$)

Mohrhauer and Holman (1963a), Rahm and Holman (1964), Tinsley (1961), and Lowry and Tinsley (1966) have demonstrated that feeding

rats increasingly higher concentrations of linolenic acid (18:3 ω3) increases the concentration of the fatty acids of the ω3 family in the liver and that the proportion of the fatty acids of the oleic (18:1 ω9) and linoleic (18:2 ω6) families are concomitantly reduced. They hypothesize that this interaction is due to the competition for enzymes necessary for elongation and desaturation within the individual families of fatty acids.

Since our pig experiment included an increasing quantity of 18:3 ω3 in the diet, the question arose as to whether this hypothesized competitive interaction actually occurred.

Trial II results were analyzed by correlation analysis and polynomial regression analysis as previously described. The quantity of menhaden oil consumed constituted the X axis, and the quantity of the 17:1 ω9 or 18:2 ω6 family fatty acid in question the Y axis.

The ω3 family fatty acids incorporated into the diet of the pigs as menhaden oil and subsequently ingested resulted in a significantly depressed deposition of the quantity of certain members of the ω6 and ω9 families of fatty acids. The mechanism involved, according to the accepted hypothesis, is that the parent fatty acids of the various fatty acid families trigger a highly competitive mechanism for the metabolic enzymes of the systems of carbon-chain elongation and dehydrogenation. Successful competition for the enzymes depends upon an affinity preference (ω3, ω6, and ω9) and upon the relative concentration of the various fatty acids, or upon both affinity and concentration. These results agree in part with the experimental evidence (Mohrhauer and Holman, 1963a) whereby the feeding of increasing levels of one of the parent acids or other members of a family results in an accumulation of acids

TABLE 24.—Liver tissue—comparison of correlation coefficients and significant degree of polynomial regression obtained by relating the quantity of menhaden oil consumed (X) until the pigs attained body weights of 90.9, 79.5, or 68.0 kg to the amount of individual fatty acids deposited in liver tissue (Y).

Fatty acid fed and deposited	Correlation coefficients when oil is fed until the pigs weighed:			Last degree of polynomial significant when oil was fed until the pigs weighed:					
				90.9 kg		79.5 kg		68.0 kg	
	90.9 kg	79.5 kg	68.0 kg	Degree	F value	Degree	F value	Degree	F value
22:6 ω3	0.69**	0.70**	0.65*	Quadratic	7.78*	Linear	11.52**	Linear	6.46*
22:5 ω3	0.78**	0.56*	0.59*	Quadratic	5.94*	Linear	5.49*	--	4.68
22:5 ω6	−0.27	−0.60*	−0.83**	--	0.92	Linear	6.73*	Linear	19.30**
22:4 ω6	−0.65**	−0.63*	−0.69*	Cubic	58.73**	Cubic	5.38*	Linear	8.08**
20:5 ω3	0.89**	0.50	0.67*	Quadratic	14.53**	--	3.98	Linear	7.45**
20:4 ω3	−0.21	−0.42	0.39	--	0.77	--	2.57	--	1.63
22:1 (?)	−0.40	−0.23	0.23	--	3.30	--	0.68	--	0.49
20:4 ω6	−0.70**	−0.19	−0.74**	Quadratic	6.62*	--	0.46	Linear	10.79**
20:3	−0.14	0.36	0.43	--	0.32	--	1.74	--	2.02
21:1 ω9	−0.59**	−0.40	−0.47	Linear	9.19**	--	2.26	--	2.56
20:2 ω6	−0.52*	−0.34	0.13	Cubic	7.59*	--	1.60	--	0.15
20:2 ω9	−0.12	−0.48	0.52	--	0.26	--	3.61	--	3.42
18:4 ω3	−0.00	−0.29	0.33	--	0.00	--	1.11	--	1.06
20:1 ω9	−0.22	−0.38	−0.28	--	0.87	--	2.04	--	0.77
18:3 ω3	0.26	−0.39	0.12	--	1.20	--	2.14	--	0.13
20:0	0.16	−0.22	0.07	--	0.47	--	0.61	--	0.05
18:2 ω6	−0.33	0.16	−0.12	--	2.10	--	0.31	--	0.13
19:0	0.10	0.03	0.43	--	0.16	--	0.01	--	2.02
18:1 ω9	−0.09	−0.49	−0.54	--	0.15	--	3.80	--	3.76
18:0	−0.06	0.33	−0.07	--	0.06	--	1.46	--	0.05
16:2	0.24	−0.30	0.47	--	1.08	--	1.20	--	2.53
17:0	0.13	0.02	0.47	--	0.31	--	0.00	--	2.48
16:1 ω7	0.02	−0.49	−0.12	--	0.01	--	3.72	--	0.13
16:0	−0.26	−0.42	−0.22	--	1.25	--	2.56	--	0.46
15:1	0.10	−0.13	0.45	--	0.17	--	0.22	--	2.25
15:0	−0.28	−0.27	0.75**	--	1.44	--	0.94	Linear	11.76**
14:1	−0.01	−0.27	0.55	--	0.00	--	0.96	--	3.84
14:0	0.01	−0.47	0.06	--	0.00	--	3.32	--	0.03

* $P < .05$ ** $P < .01$

TABLE 25.—Inner backfat tissue—comparison of correlation coefficients and significant degree of polynomial regression obtained by relating the quantity of menhaden oil consumed (X) until the pigs attained a body weight of 90.9, 79.5, or 68.0 kg to the amount of individual fatty acids deposited in inner backfat tissue (Y).

Fatty acid fed and deposited	Correlation coefficients when oil is fed until the pigs weighed:			Last degree of polynomial significant when oil was fed until the pigs weighed:					
				90.9 kg		79.5 kg		68.0 kg	
	90.9 kg	79.5 kg	68.0 kg	Degree	F value	Degree	F value	Degree	F value
22:6 ω3	0.76**	0.58*	0.49	Linear	23.54**	Linear	6.02*	--	2.87
22:5 ω3	0.77**	−0.01	0.71**	Linear	24.94**	--	0.00	Linear	9.28*
22:5ω6	--	--	--	--	--	--	--	--	--
22:4 ω6	--	--	--	--	--	--	--	--	--
20:5 ω3	0.71**	0.55*	0.71**	Linear	17.64**	Linear	5.31*	Linear	9.33*
20:4 ω3	0.72**	0.43	0.89**	Linear	18.15**	--	2.70	Linear	35.51**
22:1 (?)	--	--	--	--	--	--	--	--	--
20:4 ω6	0.03	0.09	−0.60*	--	0.02	--	0.10	Linear	4.99*
20:3	−0.02	0.11	0.13	--	0.01	--	0.15	--	0.16
21:1 ω9	--	--	--	--	--	--	--	--	--
20:2 ω6	--	--	--	--	--	--	--	--	--
20:2 ω9	−0.28	−0.25	−0.32	--	1.43	--	0.77	--	1.06
18:4 ω3	0.70**	0.15	0.54	Linear	16.65**	--	0.26	--	3.77
20:1 ω9	−0.48*	−0.31	−0.32	Linear	5.19*	--	1.25	--	0.99
18:3 ω3	0.52*	0.17	0.52	Linear	6.46*	--	0.36	--	3.30
20:0	−0.33	0.01	−0.07	--	2.01	--	0.00	--	0.05
18:2 ω6	0.41	0.10	−0.01	--	3.49	--	0.11	--	0.00
19:0	--	--	--	--	--	--	--	--	--
18:1 ω9	−0.09	−0.37	−0.46	--	0.14	--	1.96	--	2.37
18:0	−0.21	−0.13	0.17	--	0.82	--	0.20	--	0.25
16:2	0.50*	−0.03	0.50	Linear	5.96*	--	0.01	--	2.96
17:0	0.49*	0.43	0.51	Linear	5.52*	--	2.72	--	3.22
16:1 ω7	0.31	−0.17	0.08	--	1.87	--	0.37	--	0.07
16:0	−0.24	0.37	0.21	--	1.06	--	1.94	--	0.40
15:1	−0.31	0.12	0.33	--	1.77	--	0.18	--	1.13
15:0	0.13	−0.25	0.19	--	0.28	--	0.79	--	0.34
14:1	−0.18	0.27	0.35	--	0.60	--	0.91	--	1.23
14:0	−0.24	0.19	0.46	--	1.03	--	0.43	--	2.39

* $P < .05$ ** $P < .01$

derived from these acids and in a concomitant decrease in the quantity of acids of the other families.

In Trial II, we demonstrated the interrelation of fatty acid families.

Specific evidence that the deposition of the ω6 and ω9 families are inhibited appears in Tables 24 to 27. As the quantity of the ω3 fatty acids fed and deposited increased, the quantity of the 20:2 ω6, 21:1 ω9, 20:4 ω6, and 22:4 ω6 fatty acids found in the various tissues decreased significantly. The linolenic (ω3) family fatty acids of the menhaden oil therefore inhibited the conversion of oleic (18:1 ω9) and linoleic (18:2 ω6) to the elongated and dehydrogenated members of their respective families by preventing the addition of two carbons or the removal of two hydrogens, or by preventing both the addition of two carbons and the removal of two hydrogens.

SUMMARY AND CONCLUSIONS

Pigs were fed diets containing fish oil in two feeding trials to investigate (1) the organoleptic effect produced in pig tissue by feeding pigs stabilized crude menhaden oil; (2) the possible retention or disappearance of off-flavor by withdrawing the fish oil from the diet at given times; (3) the nature of the fatty acid composition of the inner backfat tissues, the outer backfat tissues, the liver tissues, and the *longissimus dorsi* tissues; (4) the relation of composition to off-flavor (if an off-flavor is produced); and (5) the hypothesized metabolic interactions of fatty acid families.

An off-flavor and a fishy flavor were detected in the meat of pigs fed diets containing about 1% of menhaden oil.

Gas-liquid chromatographic analyses of the tissues indicated that up to 28 saturated and

TABLE 26.—Outer backfat tissue—comparison of correlation coefficients and significant degree of polynomial regression obtained by relating the quantity of menhaden oil consumed (X) until the pigs attained body weights of 90.9, 79.5, or 58.0 kg to the amount of individual fatty acids deposited in outer backfat tissue (Y).

Fatty acid fed and deposited	Correlation coefficients when oil is fed until the pigs weighed:			Last degree of polynomial significant when oil was fed until the pigs weighed:					
				90.9 kg		79.5 kg		68.0 kg	
	90.9 kg	79.5 kg	68.0 kg	Degree	F value	Degree	F value	Degree	F value
22:6 ω3	0.85**	0.58*	0.74**	Linear	43.30**	Linear	6.66*	Linear	11.15**
22:5 ω3	0.89**	0.85**	0.84**	Linear	66.64**	Linear	30.88**	Linear	21.23**
22:5 ω6	--	--	--	--	--	--	--	--	--
22:4 ω6	--	--	--	--	--	--	--	--	--
20:5 ω3	0.89**	0.79**	0.74**	Linear	63.25**	Linear	19.53**	Linear	11.12**
20:4 ω3	0.76**	0.84**	0.41	Linear	23.48**	Linear	28.60**	--	1.82
22:1 (?)	--	--	--	--	--	--	--	--	--
20:4 ω6	−0.26	−0.28	−0.16	--	1.25	--	0.98	--	0.24
20:3	−0.03	−0.12	−0.15	--	0.01	--	0.17	--	0.19
21:1 ω9	--	--	--	--	--	--	--	--	--
20:2 ω6	--	--	--	--	--	--	--	--	--
20:2 ω9	−0.36	−0.44	−0.37	--	2.57	--	2.84	--	1.43
18:4 ω3	0.58**	0.36	0.18	Linear	8.63**	--	1.75	--	0.32
20:1 ω9	−0.54**	−0.32	−0.37	Linear	7.14*	--	1.37	--	1.40
18:3 ω3	0.49**	0.72**	0.08	Linear	5.45*	Linear	13.09**	--	0.06
20:0	0.57**	0.73**	0.35	Linear	8.32**	Linear	13.62**	--	1.29
18:2 ω6	0.04	0.13	−0.36	--	0.03	--	0.22	--	1.35
19:0	--	--	--	--	--	--	--	--	--
18:1 ω9	−0.24	−0.47	−0.22	--	1.04	--	3.42	--	0.46
18:0	−0.13	−0.03	0.47	--	0.27	--	0.01	--	2.56
16:2	0.44*	−0.01	0.07	--	4.18	--	0.00	--	0.05
17:0	0.50*	0.06	0.24	Linear	5.66*	--	0.04	--	0.57
16:1 ω7	0.14	0.48	−0.40	--	0.33	--	3.59	--	1.70
16:0	−0.39	0.14	0.10	--	3.00	--	0.23	--	0.09
15:1	0.26	−0.18	0.06	--	1.28	--	0.40	--	0.40
15:0	0.43	0.04	0.08	--	3.89	--	0.02	--	0.06
14:1	0.32	−0.05	0.01	--	1.96	--	0.03	--	0.00
14:0	0.18	−0.25	−0.78**	--	0.56	--	0.81	Linear	14.14*

* $P < .05$ ** $P < .01$

unsaturated fatty acids were present. Unsaturated fatty acids from four of the fatty acid families (ω3, ω6, ω9, and ω7) were found in each of the tissues.

The quantity of the characteristic fatty acids (ω3) of the fish oil fed correlated significantly and positively with the quantity of these fatty acids deposited in the inner backfat tissues and the outer ones and the liver tissues, but not in the *longissimus dorsi* tissues.

Similarly, the quantity of these ω3 fatty acids fed correlated significantly and positively with a weighted organoleptic score of the inner backfat tissues. Removal of these fatty acids from the diet of the pigs at two different body weights—namely, 68.0 and 79.5 kg—prior to their being marketed at 90.9 kg resulted in a loss of significance by the correlation coefficients, although the correlation coefficients obtained were positive.

On a practical feeding basis, fish oil with similar fatty acid composition consumption should be limited to 0.8% of the diet if fed until pigs are marketed. If the oil is withdrawn from the diet prior to marketing, higher levels can be fed.

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TABLE 27.—*Longissimus dorsi* tissue—comparison of correlation coefficients and significant degree of polynomial regression obtained by relating the quantity of menhaden oil consumed (X) until the pigs attained body weights of 90.9, 79.5, or 68.0 kg to the amount of individual fatty acids deposited in *longissimus dorsi* tissue (Y).

Fatty acid fed and deposited	Correlation coefficients when oil is fed until the pigs weighed:			Last degree of polynomial significant when oil was fed until the pigs weighed:					
				90.9 kg		79.5 kg		68.0 kg	
	90.9 kg	79.5 kg	68.0 kg	Degree	F value	Degree	F value	Degree	F value
22:6 ω3	−0.09	0.46	0.46	--	0.15	--	3.29	--	2.38
22:5 ω3	−0.22	0.75**	0.24	--	--	Linear	15.82**	--	0.53
22:5 ω6	--	--	--	--	0.85	--	--	--	--
22:4 ω6	−0.26	0.09	0.23	--	1.19	--	0.10	--	0.52
20:5 ω3	0.01	0.43	0.59*	--	0.00	--	2.73	--	4.79
20:4 ω3	−0.34	0.05	0.10	--	2.26	--	0.03	--	0.09
22:1 (?)	--	--	--	--	--	--	--	--	--
20:4 ω6	−0.58**	0.10	−0.44	Linear	8.76**	--	0.11	--	2.16
20:3	−0.22	0.27	−0.20	--	0.84	--	0.91	--	0.38
21:1 ω9	−0.32	−0.65**	0.38	--	1.96	Linear	8.70*	--	1.50
20:2 ω6	−0.46*	−0.68**	−0.21	Linear	4.56*	Linear	10.47**	--	0.41
20:2 ω9	−0.34	−0.39	−0.43	--	2.24	--	2.17	--	2.02
18:4 ω3	--	--	--	--	--	--	--	--	--
20:1 ω9	0.02	−0.45	−0.42	--	0.01	--	3.05	--	1.91
18:3 ω3	0.07	0.20	−0.23	--	0.09	--	0.51	--	0.51
20:0	−0.01	−0.05	−0.27	--	0.00	--	0.04	--	0.68
18:2 ω6	−0.25	0.53*	−0.39	--	1.15	Linear	4.77*	--	1.64
19:0	0.29	−0.02	−0.24	--	1.50	--	0.00	--	0.55
18:1 ω9	0.46*	−0.36	0.44	Linear	4.59*	--	1.77	--	2.17
18:0	0.09	−0.17	0.40	--	--	--	0.35	--	1.73
16:2	−0.04	0.11	−0.13	--	0.02	--	0.14	--	0.14
17:0	−0.05	−0.11	−0.12	--	0.04	--	0.15	--	0.14
16:1 ω7	−0.09	−0.32	0.08	--	0.13	--	1.33	--	0.06
16:0	−0.04	−0.58*	−0.16	--	0.03	Linear	6.09*	--	0.25
15:1	--	--	--	--	--	--	--	--	--
15:0	−0.22	−0.04	−0.03	--	0.89	--	0.02	--	0.01
14:1	−0.06	0.32	−0.08	--	0.06	--	1.35	--	0.06
14:0	−0.20	−0.26	−0.11	--	0.74	--	0.84	--	0.12

* $P < .05$ ** $P < .01$

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CETACEANS FROM THE LESSER ANTILLEAN ISLAND OF ST. VINCENT

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ABSTRACT

A preliminary list of cetaceans collected and observed during the course of a fishery for blackfish or pilot whales (*Globicephala*) in the waters of the Lesser Antillean island of St. Vincent is presented and includes: *Megaptera novaeangliae*, *Steno bredanensis*, *Tursiops truncatus*, *Grampus griseus*, two species of *Stenella* to which specific names are not formally applied, *Feresa attenuata*, *Pseudorca crassidens*, *Globicephala macrorhyncha*, *Orcinus orca*, *Physeter catodon*, and *Ziphius cavirostris*. Nearest published records in the western Atlantic are given, as well as limited biological notes on some of the species. The taxonomic relationships of the two forms of *Stenella* are suggested and both species are illustrated. Landings of pilot whales in the fishery over a period of 9 years are included.

There is a small but active fishery for blackfish or pilot whales (*Globicephala*) centered around the village of Barrouallie on the western or lee side of the Lesser Antillean island of St. Vincent. While the major direction of this fishery is the pursuit of blackfish, intensive studies made by the writers independently and cooperatively over the past several years have shown that a number of other small cetaceans are captured as well. The primary purpose of the fishery is the production of meat and cooking oil, both used locally, and the species taken is not especially important to the fishermen, except that the larger animals are the more profitable. Hence the concentration on blackfish. Anything that is seen is pursued except the larger and fast baleen whales. The techniques and history of the blackfish fishery have been discussed by Brown (1945, 1947), Hickling (1950), Morice (1958), Allen (1966),[5] Morris (1966),[6] Jackson (1967),[7] Rathjen and Sullivan (1970), Caldwell and Caldwell (in press), and others. In brief it is conducted from small open boats launched daily from shore and powered primarily by sail and oar (see Rathjen and Sullivan, 1970). One motor launch recently has been employed and it produces the majority of the catches of the faster swimming small dolphins. Other motor launches, both inboard and outboard, are planned (Caldwell and Caldwell, in press). The cetaceans are taken both by hand harpoon and by small gun harpoons fired from a fixed stand on the bow of the boat.

We present here a summary of our findings to date regarding species taken in the fishery. The records of odontocetes are supported by skeletal remains and/or recognizable photographs of carcasses or parts of carcasses. Copies of all of the photographs mentioned below are in the Caldwells' files with duplicates of some in those of Rathjen and Sullivan. The skeletal material, unless otherwise stated, presently is being studied by the Caldwells at the Florida State Museum, Gainesville. The "SV" numbers associated with records discussed in the text are field numbers in the Caldwells' files. With more collecting and analysis of the results,

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[5] Allen, W. O. 1966. The fishing industry in St. Vincent. St. Vincent Teachers College, Kingstown, St. Vincent, Unpublished report (file no. 50), 42 p.

[6] Morris, E. L. 1966. A brief history of Barrouallie from 1719 to present day (1966). St. Vincent Teachers College, Kingstown, St. Vincent, Unpublished report (file no. 39), 51 p.

[7] Jackson, L. R. 1967. The blackfish industry of Barrouallie, St. Vincent. St. Vincent Teachers College, Kingstown, St. Vincent, Unpublished report (file no. 26), 38 p.

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both of which are ongoing, it should be possible to say more about some of the St. Vincent faunal elements than is appropriate now. Our purpose here is only to provide an annotated summary of the existing records for this island in order to give for the first time a relatively complete list of such a fauna from one specific locality in the Antilles and to provide a firmer basis for zoogeographic statements required for ongoing studies in this and other disciplines in the West Indies and Caribbean.

Although there are a number of individual records of cetaceans from the West Indies (in part summarized by Hershkovitz, 1966), or reports which include as many as three or four species, there are few reports which include enough of the expected species to give sufficient data for evaluating the local cetacean fauna. The best of these are from the northern Antilles (from Cuba by Cuni, 1918, and Aguayo, 1954; and from Puerto Rico to Antigua by Erdman, 1970).

Studies similar to ours on small cetaceans, but more detailed, have been conducted on the Atlantic coast of Africa in the vicinity of Senegal and to some lesser degree the Ivory Coast and the Cape Verde Islands. The odontocete cetacean fauna in those similar latitudes is remarkably similar to that of St. Vincent even though the two areas lie some 5000 km apart across the open sea. This similarity extends even to forms such as *Steno* and *Feresa* that are generally rare in collections. Cadenat and others have published a series of reports on their studies of the African fauna, but a list of the species found can be had by consulting a combination of two of these (Cadenat, 1949, 1959). The latter also summarizes much of the other literature on the cetaceans of the area. Van Bree and Cadenat (1968) in addition recorded *Peponocephala electra* from Senegal.

SPECIES ACCOUNTS

With the exception of a spotted dolphin, we follow Rice and Scheffer (1968) in our arrangement of species and in the scientific and or common names applied to them.

Megaptera novaeangliae (BOROWSKI)—HUMPBACK WHALE

In early May 1968, an individual of adult size slowly passed close along the lee shore of St. Vincent in a southward direction. Several of the St. Vincent whalemen, familiar with this species from seeing it in a nearby humpback fishery at Bequia, observed this individual from boats but made no effort to harpoon it. The St. Vincent whalemen tell us that they see a few individuals of this species each year but that they never try to harpoon one because of the large size of these whales.

A few humpbacks are usually taken each year (mostly from February to April) in the Bequia fishery just to the south in the St. Vincent Grenadines which utilizes bomb guns in addition to hand harpoons. Accounts of the latter fishery were given by Brown (1945), Fenger (1958), Mitchell (1965), and Quashie (1966).[3] An early account of New England whaling vessels hunting humpbacks in the region was included by Lindeman (1880), but Clark (1887: pl. 183) for as early as 1880 included waters near St. Vincent on a map showing abandoned humpback whaling grounds. These and other reports mention *Megaptera* in St. Vincent and or nearby waters.

Steno bredanensis (LESSON)—ROUGH-TOOTHED DOLPHIN

A skull (SV-1-SB) of a specimen of unknown size and sex was obtained from the fishery in the spring of 1969. This is the first record for this species from St. Vincent and from the Caribbean. We find no prior and contemporary basis for Kellogg's (1940: 69) inclusion of this species in the Caribbean fauna, nor for the indication by Hall and Kelson (1959: 819) that its range is continuous in the western Atlantic from Virginia to South America.

The records closest to St. Vincent in the western Atlantic are from off Havana, Cuba (and thus non-Caribbean) to the north, as *S. rostratus* (see Aguayo, 1954), and from an unstated locality off the Brazilian coast to the south (Ham-

[3] Quashie, I. N. 1966. The whale industry in Bequia. St. Vincent Teachers College, Kingstown, St. Vincent, Unpublished report (file no. 30), 32 p.

ilton, 1945). *S. fuseus* Gray, from an unstated area in Cuban waters, may be this species (True, 1889: 27) and if so would represent another Cuban record. However, the unique type of this latter species, a preserved fetus, apparently was lost even in True's time and the record cannot be substantiated. Presumably the previous Caribbean records of this species (see above) have been based on this Cuban material.

Tursiops truncatus (MONTAGU)—BOTTLENOSED DOLPHIN

The Caldwells have color photographs of the head of an immature specimen of unknown size and sex taken in the fishery on 17 May 1968. The head was obtained from a market where it had been split longitudinally in order to get to the brains, which are eaten. The skull (SV-1-TT) is also split but complete.

This species has not been reported from St. Vincent before, but Turner (1912: 135) listed the mandible (as *Tursiops tursio*) of a specimen from nearby Barbados.

Grampus griseus (G. CUVIER)—RISSO'S DOLPHIN OR GRAY GRAMPUS

The Caldwells have the skull (SV-1-GG) and color photographs of the intact head of a specimen of unknown size and sex taken in the fishery in the summer of 1968. They also have the skulls of two more specimens (SV-2-GG, SV-3-GG), of unknown size and sex, taken during the summer of 1970.

In addition, the Caldwells have black and white photographs (SV-4-GG) of an individual (also of unknown size and sex) taken in September or October 1967.

Until recently this species was considered a northern form in the western Atlantic, but specimens are now available from Florida (Paul, 1968) and there is a recent sight record from the Virgin Islands (Erdman, 1970). The records from St. Vincent seem to be the most southern in the western Atlantic, and the only West Indian ones supported by specimens. Mention of this species in the St. Vincent fishery was made by Caldwell and Caldwell (in press).

Stenella, SPECIES A—LONG-SNOUTED OR SPINNER DOLPHIN

The Caldwells have color photographs of the head of a specimen of unknown size and sex taken in the fishery on 17 May 1968. The skull (SV-1-SL) was saved but is split longitudinally (see *Tursiops* account above).

The Caldwells measured three females (177.0 [SV-2-SL], 166.5 [SV-3-SL] and 150.0 [SV-4-SL] cm from tip of upper jaw to fluke notch) which were taken on 24 May 1968. They have black and white photographs (Figure 1) of the middle-sized animal taken from several angles, and the skulls of the two largest.

There is confusion in the literature regarding the systematics of the long-snouted spinning dolphins of the genus *Stenella*, and the group is badly in need of revision. The St. Vincent dolphins clearly spin, as observed by all of us at sea off the island, and our specimens (externally) and their skulls compare favorably with those reported from the northern Gulf of Mexico as *S. longirostris* (Gray) by Layne (1965), but neither we nor Layne made similar comparisons with *S. roseiventris* (Wagner) which Rice and Scheffer (1968) retained as a species separate from *S. longirostris* although many writers consider them to be synonyms. For the present we do not apply a specific name to our material but note only that the specimens from St. Vincent appear to belong to the "*longirostris-roseiventris*" group of *Stenella*.

The closest western Atlantic records for dolphins of this type are from the Bahamas near Miami, Fla., to the north (Moore, 1953) and (as *Delphinus microps*) from Brazil (no locality) to the south (Gray, 1850: 126).

Stenella, SPECIES B—SPOTTED OR BRIDLED DOLPHIN

The Caldwells measured a 172.5-cm male taken in the fishery on 24 May 1968, and have black and white photographs (Figure 2) taken from several angles and the skull (SV-1-SF). The photographs, taken under adverse lighting conditions, do not show the spotted pigmentation pattern of the specimen because in addition

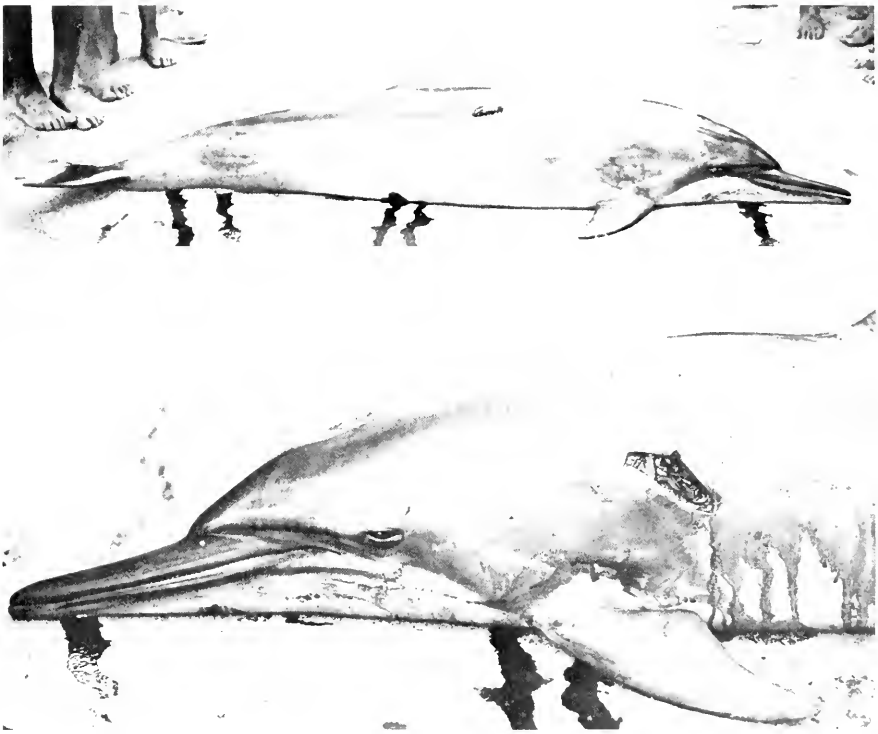

FIGURE 1.—*Stenella*, species A, 166.5-cm female spinner dolphin (SV-3-SL) landed at Barrouallie, St. Vincent, on 24 May 1968. UPPER: lateral view of entire carcass; LOWER: view of head and pectoral region showing prominent features of pigmentation. Photographs made under conditions of adverse lighting several hours after the animal had been harpooned and kept in the sun in an open boat at sea. (Photographs by William A. Huck.)

it had been in the sun most of the day and had turned essentially black as dolphins often do under such circumstances. The underlying spotted pigmentation was, however, like that in the photographs noted below.

The Caldwells also have a color lateral photograph of the anterior part of the body and one of most of the ventral side of a male, apparently an adult (SV-2-SF), taken in early June 1967. Both of these photographs show details of the spotted pigmentation, and a black and white reproduction of the one of the head clearly shows this (Figure 3).

The St. Vincent spotted dolphins we have seen seem best to fit Fraser's (1950b) and Nishiwaki's (1965) discussions and illustrations of *S. frontalis* (G. Cuvier). We tentatively would assign the St. Vincent records to that species were it not better to refrain from doing so at this time because of the already chaotic taxo-

FIGURE 2.—*Stenella*, species B, 172.5-cm male spotted dolphin (SV-1-SF) landed at Barrouallie, St. Vincent, on 24 May 1968. Photograph made under conditions of adverse lighting several hours after the animal had been harpooned and kept in the sun in an open boat at sea. (Photograph by William A. Huck.)

nomic situation in which one finds the spotted dolphins of this genus.

The Caldwells have had considerable experience in Florida with carcasses and live specimens of the spotted species *S. plagiodon* (Cope) (see D. K. Caldwell and M. C. Caldwell, 1966) and do not believe that that species as they understand it is the same as the St. Vincent form. Perrin (1970) concurred that *S. plagiodon* is separable from *S. frontalis* at least on the basis of color pattern. We concur with Mitchell (1970: pl. 5) that the spotted dolphin he pictured as having been taken from continental shelf waters near Trinidad is best assigned to the species *S. plagiodon*. Despite the relatively close proximity of the Trinidad record to St. Vincent (some 275 km), we believe that two species of spotted dolphins are involved and that Mitchell's record bears out an earlier contention by the Caldwells (D. K. Caldwell and M. C. Caldwell, 1966: 2) that *S. plagiodon* is a species found primarily in offshore waters near continents. Around the seemingly more isolated noncontinental islands of the Antilles, at least, it appears in our experience to be replaced by *S.* cf. *frontalis* or some very similar spotted species. We believe, therefore, that Rice and Scheffer (1968) were too conservative in their conclusions regarding spotted dolphins and that more than one species exists.

A mandible, reported as *Prodelphinus* sp., but probably of the same species as ours from St. Vincent, was listed from nearby Barbados by Turner (1912: 138). The next closest western Atlantic record (as *Prodelphinus froenatus*) is from southeastern Florida (Allen, 1925) to the north. It apparently has not been recorded to the south (see Hershkovitz, 1966: 36).

Feresa attenuata GRAY— PYGMY KILLER WHALE

An adult skull (SV-1-FA) of undetermined sex was obtained from the fishery in the spring of 1969. A detailed report on this specimen was prepared (Caldwell and Caldwell, 1971) as it then was the first record for the western Atlantic. After this report was accepted for publication, a record from Texas was published (James, Judd, and Moore, 1970). To our knowledge the St. Vincent specimen is still the only record from the Caribbean and West Indian region.

FIGURE 3.—*Stenella*, species B, apparently adult male spotted dolphin (SV-2-SF) landed at Barrouallie, St. Vincent, in early June 1967. Lateral view of head showing prominent features of pigmentation not shown (but present) in the animal depicted in Figure 2. Animal in freshly killed condition. (Photograph by John R. Sullivan.)

Pseudorca crassidens (OWEN)—FALSE KILLER WHALE

The Caldwells have two adult skulls, a male (SV-1-PC) and a female (SV-2-PC), from animals of unstated size taken in the fishery on 10 September 1970.

Three individuals of this species were taken in the fishery on 9 March 1969, and the Caldwells have several teeth (SV-3-PC) said to have come from one or more of these animals. Five others had been taken on 12 February and seven more were obtained on 7 December 1969.

Except for a brief mention by Caldwell and Caldwell (in press), false killer whales have not been reported from St. Vincent previously. The closest record based on a specimen is from Aves Island off the Venezuelan coast some 675 km to the southwest (Miller, 1920). Bruyns (1969) included a sight record made at sea 115 km east of Tobago (some 280 km southeast of St. Vincent).

Globicephala macrorhyncha GRAY—SHORT-FINNED PILOT WHALE OR BLACKFISH

It is upon this species that the St. Vincent whale fishery is based and it is therefore by far the most abundant species in the overall landings in the fishery. From the fishery we have eight skulls: two from males measuring about 5 (SV-1-GM) and 6 (SV-2-GM) m in total length collected in the second week of June 1967; two from females measuring about 4.5 (SV-3-GM) and 5 (SV-4-GM) m collected with the males; and four others (SV-5 to 8-GM) from adults or near adults of unknown size and sex collected in the summer of 1968.

We have seen many carcasses of fresh-caught animals and have various color and black and white photographs in our files. We also have examined many skulls on the St. Vincent beaches where the carcasses are butchered. The carcasses all have short pectoral flippers and the skulls have expanded premaxillary bones covering the maxillaries. These characters are typical of this species (see Fraser, 1950a).

A female taken on 20 May 1968 contained a male fetus measuring 69 cm in length in a straight line from the anteriormost part of the head to the fluke notch. The Caldwells did not have facilities to preserve this specimen, but have a color photograph (SV-9-GM) which shows it to be a light reddish brown.

A male measuring 4 m in total length that was taken on 21 May 1968 had several of the obligate cetacean barnacle (*Xenobalanus globicipitus*) on the trailing edge of its left flipper.

We have not examined stomach contents of pilot whales landed at St. Vincent, but the whalemen tell use that they include both squid (including very large ones) and fish.

Cyamid parasites and remoras have been observed on blackfish landed at St. Vincent but no specimens so far have been collected.

Blackfish previously have been recorded from or near St. Vincent by Brown (1945, 1947), Hickling (1950), Fenger (1958), Morice (1958), Caldwell and Erdman (1963), Allen (see footnote 5), Morris (see footnote 6), Jackson (see footnote 7), Rathjen and Sullivan (1970), Caldwell and Caldwell (in press), and others. Although specific identifications were not always given, our experience has shown that only *G. macrorhyncha* likely is involved.

Obtained from the Barrouallie Fishermen's Cooperative Society, catch statistics for the period 1962-1970 are included in Table 1. These are pilot whales landed at Barrouallie, the main whaling port of St. Vincent. The monthly var-

TABLE 1.—Landings of blackfish (*Globicephala macrorhyncha*) at the port of Barrouallie, St. Vincent, for the period January 1962, through December 1970. Precise data concerning the following variables, in part affecting the numbers of blackfish landed, are not available: weather conditions, seasonal holidays, numbers of whaling boats operating that month, and more recently, the presence or absence of engines.

Month	1962	1963	1964	1965	1966	1967	1968	1969	1970	Total	Average
January	6	9	8	15	8	6	28	4	10	94	10.4
February	7	15	37	18	5	35	85	12	15	229	25.4
March	11	32	17	19	7	15	12	6	12	131	14.6
April	6	32	42	12	46	24	49	12	21	244	27.1
May	5	57	9	22	50	41	40	53	55	332	36.9
June	15	80	48	12	41	53	21	50	28	348	38.7
July	7	70	11	18	7	21	25	12	27	198	22.0
August	2	31	20	27	12	14	48	(1)	5	[2]159	[2]19.9
September	4	55	43	24	67	16	27	(1)	22	[2]258	[2]32.3
October	4	33	26	9	30	24	44	(1)	14	[2]184	[2]23.0
November	25	10	7	4	40	17	2	24	23	152	16.9
December	5	1	7	3	10	3	6	3	0	[2]38	[2]4.8
Total	97	425	275	183	323	269	387	[3]176	232	2367	

[1] No records kept, but blackfish taken.
[2] Based on landings for 8 years only.
[3] Year incomplete.

iations reflect local weather conditions which affect the ability of the boats to go out and the whalemen to see their quarry, more than the true abundance of whales. In addition, whaling usually almost or completely ceases for approximately 2 weeks before and 2 weeks after Christmas, in observance of the holidays. We are told that the blackfish are there year round.

Orcinus orca (LINNAEUS)—KILLER WHALE

On 13 May 1968, two females (an adult [SV-1-OO] and subadult [SV-2-OO] of unknown sizes) and one juvenile male (SV-3-OO of unknown size) were harpooned from a pod of six animals. The young male was harpooned first, and the other animals reportedly stood by (see M. C. Caldwell and D. K. Caldwell, 1966, regarding this kind of behavior) just as the St. Vincent whalemen report the blackfish in that fishery often do when certain ones of their kind are harpooned. We have several color photographs of the carcasses which show the striking black and white color pattern characteristic of this species. The skulls of the three harpooned specimens are now in the possession of the Caldwells.

Sullivan noted that one of the larger animals had several cyamid parasites on the skin, but specimens were not collected. His photographs show only that the parasites are cyamids but as Leung (1967) did not include *Orcinus* among the known hosts for cyamids the observation is noted. Caldwell and Caldwell (1969) reported that all three of these whales had eaten leatherback sea turtle (*Dermochelys coriacea*).

Three additional killer whales, including a 6-m male with very heavily worn teeth, were taken from a school of eight on 11 July 1968, but no specimens or photographs are available. One of the animals reportedly had cyamid parasites. The school was said to have been moving north about 10 km off the lee shore of the island. The fishermen's records show that four more were taken on 4 June 1969.

Jackson (see footnote 7) noted that killer whales are taken in the St. Vincent fishery and included photographs of carcasses being butchered on shore. Rathjen and Sullivan (1970) and Caldwell and Caldwell (in press) also noted the occasional capture of individuals of this species by the St. Vincent whalemen.

These records are the southernmost in the western North Atlantic for killer whales. Moore (1953) listed this species from off Miami, and Backus (1961) from the Bahamas. Erdman (1970) included several sight records from the general vicinity of the Virgin Islands. In the western South Atlantic, killer whales have not been reported north of Buenos Aires (as the type of *Orca magellanica* Burmeister—see Hershkovitz, 1966: 84).

Physeter catodon (LINNAEUS)—SPERM WHALE

The Caldwells have the prepared lower jaw (SV-1-PCA) and color photographs of the entire carcass of a juvenile female (ca. 8 m in total length) taken in the fishery on 23 May 1968. The teeth were just beginning to erupt and their pulp cavities were completely open. Mention of the stump of the left pectoral flipper of this animal, possibly missing as the result of a shark bite, was made by Wood, Caldwell, and Caldwell (1970), and a photograph of the carcass on the beach was included by Caldwell and Caldwell (in press).

The Caldwells have partial sets of mandibular teeth from two other sperm whales taken in the fishery prior to 1968. In one set (SV-2-PCA) the pulp cavities are fully open, while in the other set (SV-3-PCA), actually smaller teeth, the cavities are fully closed.

The fishermen's records show the capture of three sperm whales on 19 April 1967, two on 2 January 1969, and two on 25 April 1969 (a third was harpooned on this latter occasion but was lost with the boat). Mr. Griffith Arrindell, a leader of the St. Vincent whale fishery, told us that sperm whales are seen most commonly in the region from October to late spring, although some appear to be present year round.

Jackson (see footnote 7) and Rathjen and Sullivan (1970) mentioned that sperm whales are sometimes taken in the St. Vincent fishery and the latter writers included a photograph of the head of an 8-m male. Townsend (1935: chart B) showed sperm whales between St. Vincent and Barbados in January and February, Brown (1942) noted that a few sperm whales once were taken off the lee (western) side of Barbados (toward St. Vincent) and Brown (1945) and Fenger (1958: 44) recorded the fact that this species sometimes is taken in the nearby waters of Bequia and other islands of the Grenadines. Clark (1887: pl. 183) showed active sperm whaling grounds, fished by New England whalers, all around the southern Lesser Antilles in 1880, and this whaling continued thereon into the first part of the 20th century.

Ziphius cavirostris G. CUVIER—GOOSE-BEAKED WHALE OR CUVIER'S BEAKED WHALE

The Caldwells have color photographs of the head of a female (SV-1-ZC) and somewhat longer views of the carcass of her nearly term fetus calf (SV-2-ZC) of undetermined sex taken in the fishery in late summer 1967. Although it was recently reported from nearby Barbados (Caldwell, Rathjen, and Caldwell, in press), this species has not been reported previously from St. Vincent.

UNRECORDED BUT EXPECTED SPECIES

Both the descriptions given us by the whalemen and distributional records from other localities in the region lead us to expect that several additional species eventually will be recorded from St. Vincent. It is beyond the scope of the present preliminary report to discuss these, but a summary of all known records of marine mammals from the West Indies and Caribbean that is in preparation by the Caldwells suggests that the list from St. Vincent might be expected to include any of several species of *Balaenoptera*, *Stenella caeruleoalba*, *Delphinus*, and *Mesoplodon*. *Kogia* and *Peponocephala* might also be expected, but the present suggestive evidence is not as strong.

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A number of people at Barrouallie, the principal and most active whaling port on St. Vincent, have been of tremendous help in our study in providing observations from their own direct experience, access to records of the Barrouallie Fishermen's Cooperative Society, and in the collection and shipping of specimens. In this regard Griffith Arrindell has given us unselfish and unswerving assistance despite our constant pressure and questions. Conrad Francis and William O'Garro of that town also have given much of their energy. Partial financial support for certain phases of the study has come to DKC from the American Philosophical So-

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CONTRIBUTIONS TO THE BIOLOGY OF THE ROYAL RED SHRIMP, *Hymenopenaeus robustus* SMITH[1]

WILLIAM W. ANDERSON[2] AND MILTON J. LINDNER[3]

ABSTRACT

The royal red shrimp, *Hymenopenaeus robustus*, has been located in commercial concentrations in three areas off the coast of the United States in depths from about 250 to 550 m: one area, known as the St. Augustine Grounds, is off the east coast of Florida; another is off the Dry Tortugas; and the third is off the Mississippi River Delta.

Information on the biology of the species on the St. Augustine Grounds was collected intermittently from 1957 to 1967.

The reproductive systems of males and females are described and illustrated. The ovaries of ripe females are dark red or maroon, and the exceedingly large spermatophores are bright yellow. We observed no indication of sex reversal.

Burrowing and swimming habits as observed from the research submarine *Aluminaut* are summarized.

The early life history of *H. robustus* is unknown. Neither larval nor postlarval stages were encountered in the plankton collections of the M/V *Theodore N. Gill*. Juveniles under 50 mm total length were not caught.

Size of shrimp was not correlated with depth but appeared to be correlated with latitude. Usually shrimp were larger north of lat 29°39′ N than between lat 29°00′ and 29°39′ N.

Males mature at about 125 mm and females at about 155 mm total length. In each sex, maturity is reflected by a change in the regression of carapace length on total length.

Spawning probably occurs throughout the year, but the peak is between January and May.

Year classes are evident in the length distributions. Recruitment on the fishing grounds begins when the shrimp are approaching 1 year of age and are less than 100 mm total length. They reach maturity at about 3 years, and minimum life span appears to be no less than 5 years. Recruitment is probably not complete until at least 2 years. Most of the shrimp on the fishing grounds are mature.

The royal red shrimp, *Hymenopenaeus robustus* Smith, a large deepwater penaeid (Figure 1), has a wide distribution from the east coast of the United States to well down the east coast of South America, principally in depths of 256 to 549 m (140-300 fm).

Surveys by the Bureau of Commercial Fisheries (now the National Marine Fisheries Service) have indicated three major concentrations of these shrimp off the coast of the United States that have commercial possibilities: (1) off St. Augustine on the east coast of Florida (Figure 2), (2) off the Dry Tortugas in the Florida

FIGURE 1.—Adult female and male royal red shrimp, showing great difference in size of sexes. Female (upper) 210 mm and male (lower) 160 mm total length.

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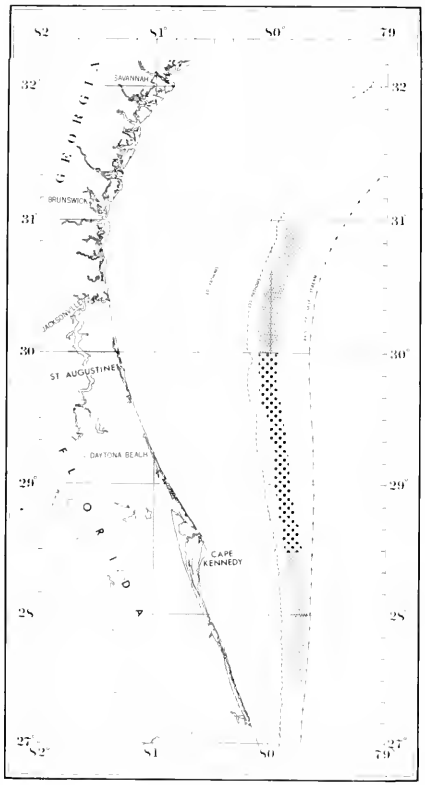

FIGURE 2.—Royal red shrimp grounds off east coast of Florida. Large dots, lat 28°30′ to 30°00′ N, represent most productive portion.

Straits, and (3) off the Mississippi River Delta. Accounts of these surveys were given by Springer and Bullis (1952, 1954), Bullis (1956), Bullis and Rathjen (1959), Bullis and Thompson (1959), Cummins and Rivers (1962), and Bullis and Cummins (1963). Anderson and Bullis (1970) gave an account of direct observations made on the St. Augustine grounds during a dive with the research submarine *Aluminaut* on September 21, 1967; Klima (1969) gave the length-weight relation; and Roe (1969) summarized the distribution on the three major grounds off the southeastern United States.

Biologists at the former BCF Biological Laboratory, Brunswick, Ga., studied the biology of royal red shrimp at the grounds off St. Augustine, Fla., by accompanying vessels of the BCF Exploratory Fishing and Gear Research Base, Pascagoula, Miss., and of the Exploratory Fishing Station, Brunswick, Ga. The work continued from 1957 to 1967, as opportunities arose, on the exploratory fishing vessels *Combat*, *Silver Bay*, and *Oregon*.

This paper presents the results of these 11 years of intermittent data-gathering.

THE ST. AUGUSTINE GROUNDS

The part of these grounds generally fished extends from about lat 28°30′ N to 30°00′ N in 256 to 475 m (140-260 fm) but the most productive part is between lat 29°00′ N and lat 30°00′ N, and it is from this area that most of the data were obtained.

The BCF surveys of the St. Augustine Grounds indicate that they average about 15.4 km (8.3 nautical miles) in width and have a steep angle of descent. The bottom between 183 and 256 m (100 and 140 fm) is largely untrawlable owing to dense stands of deep-sea alyconarians (sea fans) and limestone formations. Between 256 and 475 m (140 and 260 fm) the bottom is largely sand or silty-sand sediments (referred to as "green mud" by fishermen), is relatively free of obstructions, and provides excellent trawling conditions. Deeper than 475 m (260 fm), extensive patches of deep-sea coral, *Lophelia prolifera*, make the bottom hazardous to trawling with standard shrimp gear.

Anderson and Bullis (1970) made the following observations from the *Aluminaut* in 457 to 274 m (250-150 fm), and between lat 29°10′ and 29°20′ N: "The bottom was remarkedly free from obstructions and consisted of a grayish, loosely constituted sediment that readily clouded the water at the least disturbance. It was

formed into a myriad of shallow depressions and mounds, pitted with holes. Claws protruding from many of these holes indicated the richness of the crustacean bottom fauna. Some fishes were also observed in holes, but these were not nearly so numerous. From the bottom port it was possible to see directly into some of the holes, and we observed animals that would have been invisible at an angle."

These fishing grounds are directly under the Gulf Stream.

DATA AND METHODS

The data were obtained from operations of several vessels either owned or leased by the BCF—M V *Combat*, a 30-m converted minesweeper; M V *Silver Bay*, a 29-m New England-type trawler; and the R/V *Oregon*, a 30-m trawler. Bullis (1956) and Bullis and Rathjen (1959) have given details of vessels, gear, and operating procedures.

The data consist of length measurements and observations on ovarian development taken from random samples (100 specimens, if this number or more were caught; the total catch if fewer were caught) at irregular intervals from 1957 to 1967 (Table 1). They also consist of one set

TABLE 1.—Cruise dates, number of stations, and number of *H. robustus* in samples.

Vessel	Cruise dates	Stations sampled	Shrimp measured	
			Males	Females
		Number	*Number*	*Number*
Combat	Apr. 26-28, 1957	7	495	624
Combat	May 29-31, 1957	3	187	373
Combat	July 17-30, 1957	9	350	456
Combat	Aug. 13-20, 1957	18	798	874
Silver Bay	Nov. 20-25, 1957	14	441	755
Silver Bay	June 11-22, 1958	28	1,136	1,811
Silver Bay	Jan. 18-28, 1960	13	408	356
Silver Bay	Apr. 30-May 3, 1960	10	379	463
Silver Bay	Apr. 28-May 1, 1961	12	489	601
Silver Bay	Jan. 16-Feb. 22, 1962	24	881	1,386
Silver Bay	Aug. 22-28, 1962	10	393	525
Silver Bay	Sept. 25-28, 1962	8	373	376
Silver Bay	Feb. 5-7, 1964	6	290	309
Oregon	Nov. 11-18, 1964	21	863	1,233
Oregon	July 20-22, 1967	6	251	299
Total		189	7,734	10,441

of total length-carapace length measurements from nonrandom samples taken during July 1957. Date, location, depth (with two exceptions), and duration of haul are known for each trawling station from which the samples were taken. Data on bottom temperatures are not available. The location of each trawling station was obtained by sonar fixes and the depth, in fathoms, from sonic depth recordings. The depths have been converted to the nearest meter.

Throughout almost every cruise during which length measurements were taken, the operation simulated commercial fishing; consequently the shrimp measured were caught by several types of trawls in a wide range of sizes. Mesh sizes, however, probably did not vary much. The most commonly used trawl was constructed of "commercial" nylon webbing with 2-inch (50.8-mm) stretched mesh in the body and 1½-inch (38.1-mm) stretched mesh in the cod end. These sizes of mesh are generally used by commercial shrimp fishermen. As shown by Berry and Hervey (1965), the greatest inside dimensions usually are somewhat less than 50.8 mm and 38.1 mm, respectively. Measurements we made on the greatest inside mesh dimensions of the shrimp trawls used by the exploratory vessels for capturing *H. robustus* indicate a range between 43 and 48 mm (mean 45.7 mm or 1.8 inches) for the "commercial" 50.8-mm mesh and between 30 and 34 mm (mean 31.7 mm of 1¼ inches) for the "commercial" 38.1-mm mesh.

Mesh selectivity studies have not been made on *H. robustus*, but we doubt that the variability in types and sizes of trawls appreciably affected the lengths of shrimp caught. We assume that mesh selectivity would be about the same as that shown by Berry and Hervey (1965) for *Penaeus aztecus* for 2-hr tows. Most of our tows lasted 2 to 4 hr, with 3-hr tows predominating. The 50% escapement length for 31.7-mm mesh would thus be about 70 mm total length and specimens of *H. robustus* less than 50 mm total length, if present, would have been represented in the catches. We can expect, however, that specimens less than about 100 mm total length probably were not represented in their true perspective in the random samples.

Total length measurements (tip of rostrum to end of telson—all length measurements are

total length unless otherwise indicated) were made in ½-cm units on a measuring board so adjusted that when the ½-cm units were converted to millimeters the midpoints fell on 3 and 8 (e.g., a length of 25 ½-cm units represents lengths between 121 and 125 mm with midpoint at 123 mm; similarly, the midpoint in millimeters for 26 ½-cm units is 128 mm). Carapace lengths (orbital angle to mid-dorsal end of carapace) were measured with calipers to the nearest millimeter. Both total length and carapace length measurements were made of freshly caught specimens.

The ovarian stages, determined by visual inspection at the time each female was measured, were based on size and color of the ovaries. We also noted whether spermatophores were attached to females.

SYSTEMATICS

In the shrimp family Penaeidae the royal red shrimp, *Hymenopenaeus robustus* Smith, belongs in the subfamily Solenocerinae, which is distinguished from the three other subfamilies by having a postorbital spine (Figure 3).

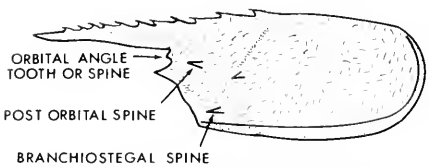

FIGURE 3.—Outline of carapace of *H. robustus* showing position of spines.

Three genera (*Haliporus* Bate, *Hymenopenaeus* Smith, and *Solenocera* Lucas) make up the subfamily Solenocerinae. *Solenocera* is distinct from the other two genera in having the antennular flagella flattened or hollowed out—channellike in structure—rather than cylindrical and filiform. *Hymenopenaeus* has a single pair of lateral telson spines and lacks podobranchs behind segment VIII, whereas *Haliporus* has several pairs of lateral telson spines and podobranchs posterior to segment VIII.

Within the genus *Hymenopenaeus*, three species (*H. robustus*, *H. modestus* Smith, and *H. lucasii* Bate) are separated from all other species by the following combination of characters: branchiostegal spine present, pterygostomian spine absent, and no postrostral teeth separated from the rostral group (Figure 3).

H. robustus is distinct from *H. modestus* and *H. lucasii* in having a tooth or spine in the orbital angle (Figure 3).

BIOLOGY OF THE SHRIMP

REPRODUCTIVE SYSTEMS

Internal

The internal reproductive organs of royal red shrimp are so similar to those described by Angelescu and Boschi (1959) for *Hymenopenaeus muelleri* and by King (1948) and Young (1959) for the white shrimp, *Penaeus setiferus*, that only gross anatomy is given here.

The ovaries are paired. In the cephalothoracic region they are partly fused; each ovary has an anterior pointed lobe and 6 to 8 fingerlike lateral projections which lie over the hepatopancreas. A lobe from each ovary extends nearly the full length of the abdomen dorsolateral to the intestine. The oviducts lead to genital pores at the bases of the third pereiopods (Figure 4).

The testes are also paired and occupy a position in the cephalothoracic region similar to that of the ovaries. Each testis has several lateral lobes, and a looped vas deferens which connects to the terminal ampoule on the coxa of the fifth pereiopod. The testes do not extend into the abdomen.

External

Details of the structure of the thelycum in the female (considered to be modifications to the sternal plates of somites XII, XIII, and XIV) are shown in Figure 4. Note the bristly, cuplike paired openings of the oviducts at the bases of the third pereiopods; the rectangular plate with

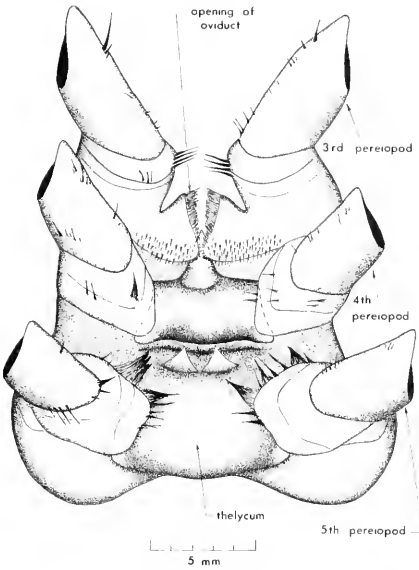

FIGURE 4.—Ventral view of thorax of adult female.

a forward projecting cone-shaped protuberance which lies between the fourth pereiopods; the paired triangular protuberances about midway between the fourth and fifth pereiopods; and the dome-shaped area between the fifth pereiopods.

Burkenroad (1936) described in detail the petasma of *H. robustus* but did not illustrate it. Figure 5 shows the petasma of *H. robustus*, spread open to show its structure.

Spermatophore

Compared with the spermatophores of the white shrimp (*Penaeus setiferus*), the brown shrimp (*P. aztecus*), and the pink shrimp (*P. duorarum*)—all penaeid shrimp similar in size to *H. robustus*—the spermatophore of the royal red shrimp is exceptionally large. In fresh material the spermatophore is bright yellow.

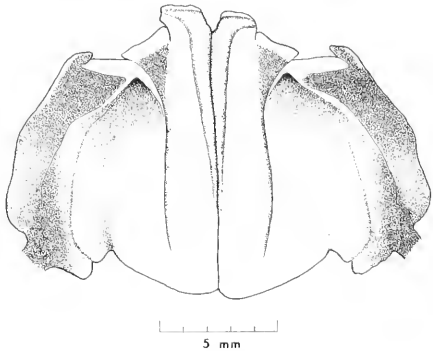

FIGURE 5.—Petasma of male spread open to show arrangement of rods and folds.

Figure 6 shows a spermatophore in attached position; Figure 7 shows a detached spermatophore. The winglike protuberances extend between the pereiopods, and the knobby and bristly sections of the coxae help hold the spermatophore in place. A gluelike substance that accompanies the spermatophore when attached by the male also helps hold it secure. The spermatophores of royal red shrimp are much more securely attached than those of the white shrimp and are not easily dislodged.

HABITS

Anderson and Bullis (1970) contributed most of our limited knowledge of the habits of this deep-sea shrimp. Their observations from the submarine *Aluminaut* were as follows: "Bottom photographs had previously indicated that royal-red shrimp stayed on the sea-floor surface, but we saw numerous shallow furrows (1 to 3 ft long) in the bottom in which royal-red shrimp were partly buried. They apparently do not burrow as deeply or completely as do brown and pink shrimp. We believe the shrimp plow into the bottom in search of food rather than protection, and that this feeding activity produces the grooves or furrows.

"When disturbed, the royal-red shrimp rise gently from the furrows and swim in normal

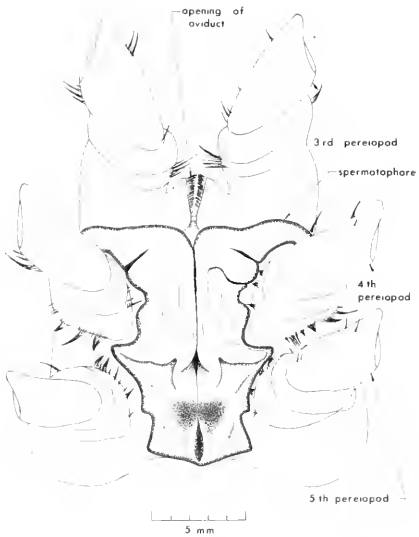

FIGURE 6.—Ventral view of thorax of adult female with spermatophore (shaded) attached.

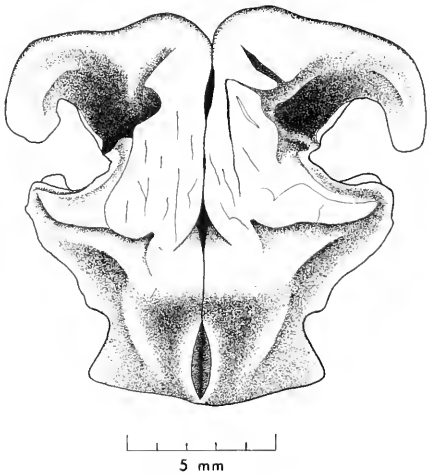

FIGURE 7.—Ventral view of detached spermatophore.

upright position. If frightened, they flip back in typical penaeid fashion by quick flexing of the abdomen and then swim forward rapidly, but usually they are turned on their sides so that they bounce off the bottom every few feet. At the end of the run they stand on the bottom rather than burrow in. The shrimp walk either forward or sideways. Color varied from grayish pink to red—similar to colors observed on trawl-caught specimens."

LARVAL, POSTLARVAL, AND JUVENILE STAGES

The larvae of *Hymenopenaeus robustus* are unknown. Several attempts were made by Anderson to hatch eggs from ripe females bearing spermatophores. The eggs failed to develop, however—perhaps because of the drastic changes in temperature and pressure when the animals were quickly brought from the cold waters of about 385 m (200 fm) to the warm surface waters of the Gulf Stream.

In attempts to find larval and postlarval royal red shrimp, we examined numerous plankton samples from the M/V *Theodore N. Gill* cruises, covering all seasons, in an area from about the 183-m (100 fm) contour to well beyond the axis of the Gulf Stream and over the entire length of the St. Augustine Grounds. Only a few larval or postlarval penaeids were found and only one of these, a *Solenocera*-like mysis stage, was considered as possibly being *Hymenopenaeus* (Harry L. Cook, then a fishery biologist at the BCF Biological Laboratory, Galveston, Tex., kindly made the identifications).

Burkenroad (1936) has provided the only record of postlarval *H. robustus*. He described nine specimens (all dead when examined), which he believed to be juveniles (postlarvae), that were collected in the northern Gulf of Mexico off the mouth of the Mississippi River. Eight (12.0-21.5 mm total length) were taken at R/V *Atlantis* station 2377 on March 24, 1935, and one (no length given) was taken at *Atlantis* station 2381 on March 26, 1935.

LATITUDE AND DEPTH DISTRIBUTIONS

A preliminary examination of our data suggested that royal red shrimp tended to be larger in the northern part of the collection area than in the central part, and that size was inversely related to depth. Correlations were significant between median lengths of females and latitude ($r = +0.46$; $t = +6.65$; $Y = 23.39 + 0.09X$) and between median lengths of females and depth ($r = -0.46$; $t = -6.65$; $Y = 53.78 - 0.11X$). The data used for these correlations were from the 164 stations at which 20 or more females were measured; the lengths are in ½-cm units. We did not repeat the calculations for the males because when the smaller size groups of females were caught in a tow, invariably the smaller size groups of males also were caught.

For the latitude correlation we grouped the median lengths by 10′ intervals of latitude. A graph of the data showed that female shrimp tended to be smaller between lat 29°00′ and 29°39′ N than between lat 29°40′ and 30°13′ N. We had samples from only five stations south of lat 29°00′ N, of which four were between lat 28°00′ and 28°39′ N. Although the data suggested that large shrimp also tended to inhabit the southern part of the grounds, we are unwilling to draw conclusions for the area south of lat 29°00′ N from this small sample.

The size and latitude relation confirmed reports of the fishermen that usually, but not always, they encountered larger shrimp on the northern portion of the grounds than on the central portion. The fishermen rarely fish the southern part of the grounds, and we received conflicting reports on the size of shrimp caught in this area.

For the depth relation we grouped the data by 25-fm (46-m) depth intervals (151-175; 176-200, etc.). The highly significant negative relation between size and depth (large shrimp in shallow water and smaller shrimp in deeper water) did not agree with some of our data (Table 2) nor with reports from the fishermen and exploratory fishing personnel; hence we suspected that this relation and perhaps that with latitude were fortuitously caused by the fishing pattern at the times our samples were taken. We therefore re-examined the data and selected only those cruises during which more than one depth class was fished in the same latitude zone and those cruises during which the same depth class was fished in the two latitude zones. The depth classes used were 150 to 175 fm (274-320 m), 176 to 200 fm (322-366 m), 201 to 225 fm (368-411 m), and 226+ fm (413+ m). The latitude zones chosen were lat 29°00′ to 29°39′ N and 29°40′ to 30°13′ N. We had no stations south of lat 29°00′N that met the requirements.

TABLE 2.—Median length of *H. robustus* and depth of hauls for eight stations in the same latitude—*Silver Bay* cruise September 25-28, 1962.

Lat N	Depth of hauls		Median length	
			Males	Females
	m	fm	mm	mm
29°54′	324-329	177-180	138	178
29°54′	329-338	180-185	138	178
29°54′	348	190	138	178
29°54′	357-362	195-198	138	178
29°53′	366	200	138	183
29°53′	375	205	138	178
29°53′	384	210	138	173
29°53′	411-421	225-230	138	178

After the selection was made, we had 12 cruises with 93 stations and 2 depth classes that could be compared by latitude zone for the same depth class; and 18 cruises with 95 stations and 4 depth classes that could be compared by depth class for the same latitude zone. Only those stations were selected from which 20 or more females were measured. The results are shown in Figures 8 and 9 and in Appendix Tables 1 and 2.

In Figure 8 we have plotted the mean median lengths (in ½-cm units) as scatter diagrams against depth class with latitude zone lat 29°00′ to 29°39′ N as the abscissa and latitude zone lat 29°40′ to 30°13′ N as the ordinate. If no relation existed between the length of the shrimp and the latitude, the dots and crosses would be scattered along and on either side of the 45° lines. All 12 marks fall above the 45° lines, however, showing that shrimp tended to be larger north of lat 29°39′ N than in the area between lat 29°00′ and 29°39′ N.

In Figure 9 we have plotted the mean median lengths as scatter diagrams separately for each

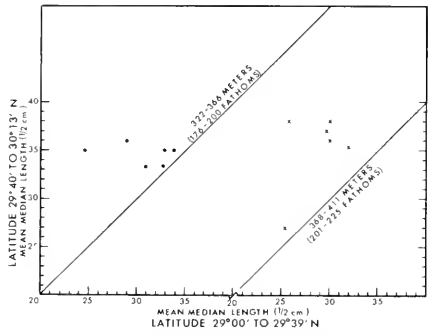

FIGURE 8.—Mean median length of females by latitude and depth (see text for explanation).

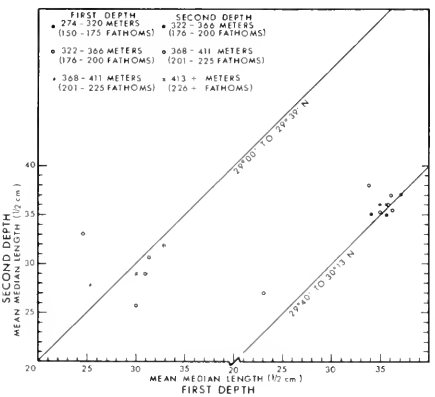

FIGURE 9.—Mean median length of females by depth and latitude (see text for explanation).

latitude zone, with the first or shallowest depth class as the abscissa and the next succeeding depth class as the ordinate. The marks for each latitude zone appear to fall in a random fashion about the 15° lines; consequently there was no apparent relation between the length of the shrimp and the depth from which they were caught. This figure also indicates that the average size of shrimp is usually, but not always, larger in that part of the St. Augustine Grounds north of lat 29°39′ N than between lat 29°00′ and 29°39′ N.

We are not certain why shrimp tend to be smaller in the central than in the northern portion of the St. Augustine Grounds unless most of the recruitment is in the central area. If shrimp also are larger in the southern portion of the grounds (as our meager data from that area suggest), the obvious conclusion is that most of the recruitment occurs in the central portion of the grounds; otherwise, it may be that recruitment is from the south.

SIZE AT MATURITY

The terminal ampoules were small and poorly developed in most males less than about 125 mm long but were large and prominent in specimens greater than this length. This observation suggests that sexual maturity in the males is reached at about 125 mm total length.

Development of the ovaries began in most females at about 136 mm total length, but maturity was not attained until they were about 155 mm long. In the random samples, all of the 1,327 females that had attached spermatophores were ripe. Of this number only 55 or about 4% were less than 151 mm long. The smallest ripe shrimp with attached spermatophores (three individuals) were in the 136- to 140-mm size class.

Lindner and Anderson (1956) demonstrated that female *Penaeus setiferus* underwent certain morphometric changes on attaining maturity. It is evident from Figures 10 and 11 and Appendix Table 3 that the regressions of carapace length on total length for both male and female royal red shrimp also show changes in slope at about the lengths at which each sex reaches maturity. Male royal red shrimp (Figure 10) demonstrate a change in slope at the midpoint of the 126- to 130-mm class interval, which agrees with field observations on the size at which the males reach maturity.

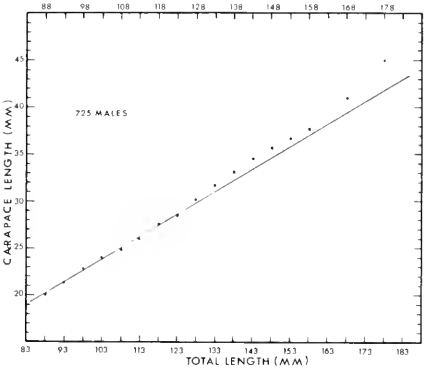

FIGURE 10.—Regression of carapace length on total length for males.

For female *H. robustus* (Figure 11) the break occurs at the midpoint of the 151- to 155-mm class interval and the new slope is not reached until the midpoint of the 161- to 165-mm class interval. Here again this morphometric change is associated with maturity.

These regressions were made from data gathered in July 1957. Possibly the lengths at which the slopes change would be different at other times of the year.

SPAWNING

It was possible to separate several stages of ovarian development in the field without microscopic examination, because maturation is accompanied by changes in size of the ovaries and by very distinct color changes. In the field, however, we were unable to distinguish with certainty the spent females. These were included in the "undeveloped" and "developing" categories. We usually recorded ovary development in the following stages:

1. UN = undeveloped. In this stage the tiny ovaries are almost threadlike and transparent.
2. D = developing. The ovaries have increased markedly in size and are opaque but have not developed a distinctive color.
3. P = pink. The ovaries continue to increase in size and first take on a light yellow color which rapidly becomes light pink.
4. R = ripe. Now swollen to full size, the ovaries are a dark red or maroon color. At this stage the male attaches the spermatophore to the female.

The length distribution of shrimp with pink ovaries differed little from the length distribution of those with ripe ovaries. Because we have no conception of the time required for the pink stage to develop to the ripe stage, and because the sizes were similar, we grouped these two ovarian stages and called them ripe. We also grouped females with undeveloped and developing ovaries, mainly to avoid discarding the data collected during the first two cruises in 1957, when these two stages were not differentiated.

We have presented this material in Figure 12 by seasonal periods. The periods chosen were November; January and February; April, May, and June; and July, August, and September.

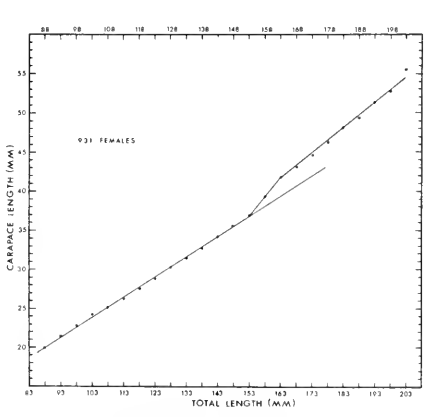

FIGURE 11.—Regression of carapace length on total length for females.

Because many more shrimp were measured during some cruises than during others in the same season, we weighted the data in Figure 12 and Appendix Table 4 to give each cruise equal weight, irrespective of the number of shrimp in the samples. Figure 12 demonstrates that the peak of spawning is during the winter and spring. Spawning probably is not extensive before December and is essentially completed by June, although some spawning continues throughout the year. Figure 12 also indicates that few females less than 150 mm long have ripe ovaries.

The occurrence of small specimens reported by Burkenroad (1936) in March corresponds with our estimate of the peak spawning season.

AGE CLASSES

In compiling total length distributions for males and females, we have again given equal weights to data from each cruise irrespective

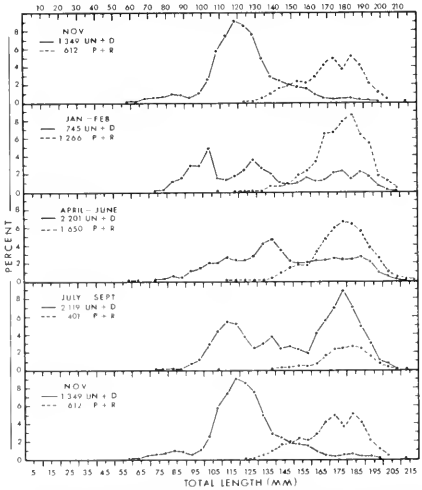

FIGURE 12.—Seasonal length distributions of female *H. robustus* by ovarian stages. (UN = undeveloped; D = developing; P = pink; R = ripe.)

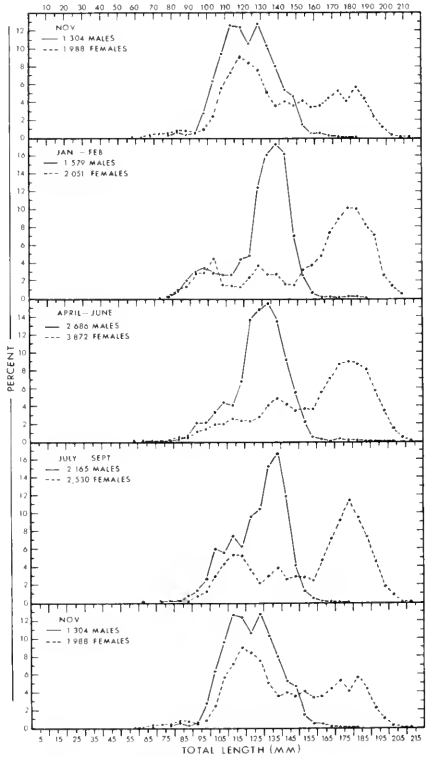

FIGURE 13.—Length distributions of *H. robustus* by sex and season.

of the number of shrimp measured, and we present the data for the same seasonal periods used to show the spawning season (Figure 13 and Appendix Table 5). The relative heights of the modes can be misleading because the data are scattered sparsely through 11 years and the appearance of a dominant year class in one sample (as occurred in November 1964) can have a disproportionate effect when applied to the material as a whole.

The graphs for the females are more readily interpreted than those for the males. In No-

vember the females show a mode at about 80 mm total length that can be followed readily throughout the year to a second mode at about 120 mm. The mode at 120 mm can also be traced throughout the year to the bimodal group with modes at 143 and 153 mm. The bimodality of this latter group we believe to be fortuitous, but it can be followed to a hump between 153 and 158 mm in the January-February distributions. Thereafter the modes become lost in the large group of mature females. We believe that the two additional modes in our November distributions (173 and 183 mm) also are fortuitous and result from the sampling procedures.

A group of small male shrimp also appears in November, forming a mode at about the same length as that for the females, which can be traced throughout the year to the second mode at about 115 mm. This second mode can be traced as a hump to the left of the main mode from January through September to the third mode at 128 mm, beyond which it is lost.

We made various attempts to fit Von Bertalanfy growth curves to the data without satisfactory results other than it was apparent the first two modes could be attributed to 1- and 2-year-old shrimp. Evidently morphometric changes associated with maturity preclude the use of total length as a means of determining age of *H. robustus* after they reach maturity.

We believe the first three groups of males and females we have cited are 3 distinct age classes. If the peak of spawning is in March, both sexes would be about 100 mm total length at 1 year of age. From our data it is impossible to distinguish more than the first 3 age classes. The older age classes, which probably represent 2 or more additional years, would give a minimum life span of 5 years. Probably, however, at least some of the largest shrimp are older than this.

SIZE AND AGE AT RECRUITMENT

When we consider all of our length measurements as a unit, either unweighted, weighted to give each cruise equal weight, or weighted to give each year equal weight, we obtain almost identical distributions. In Figure 14 we show

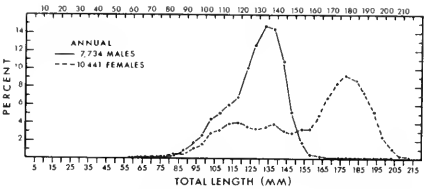

FIGURE 14.—Length distributions of *H. robustus* by sex for all samples combined.

these length distributions weighted to give equal weight to collections during each cruise. It is readily evident from these curves that only the groups presumed to represent the second, third, and fourth and older age groups are present in substantial numbers and that the population is composed largely of mature shrimp.

Although the data are not adequate, they suggest that recruitment starts at about 1 year of age, but the shrimp are not fully recruited until about 2 years of age, and recruitment may not be complete until the shrimp are mature—about 3 years old. In the combined length distributions (Figure 14), 55% of the females were longer than 160 mm and 61% of the males were longer than 125 mm (the lengths at which we believe each sex is fully mature). Only 6% of the males and 4% of the females were less than 100 mm long. The smallest shrimp we sampled was in the 56- to 60-mm length class. As we mentioned earlier, we do not believe that gear selectivity causes the scarcity of shrimp under 100 mm long and the lack of them under 56 mm long. Royal red shrimp do not appear on the fishing grounds at sizes smaller than about 55 mm. The observations of Anderson and Bullis (1970) who had clear visibility of the bottom from a distance of less than 1 m, substantiated the lack of small shrimp on the St. Augustine Grounds. Furthermore, 37 *H. modestus*, 43 to 93 mm long (mode, 63 mm) were taken on April 20, 1957, in 225 fm with a 40-ft flat shrimp trawl fitted with commercial 2-inch stretched mesh in the body and 1½-inch mesh in the cod end. In addition, H. R. Bullis, BCF Pascagoula, Miss. (personal communication), concerning trawling on the *H. robustus* grounds

off the Mississippi River Delta, stated, "Of special interest was the discovery of high densities of small red (*Hymenopenaeus debilis*) shrimp in 208 fathoms. These shrimp averaged less than 35 mm total length." These two species of *Hymenopenaeus* are similar in shape to *H. robustus;* consequently, we believe that small *H. robustus* would have been taken in our gear if they had been present on the fishing grounds. We have no idea where they might be.

The sex ratios for all the data combined show 42.6% males and 57.4% females. Some, but probably not all, of this difference is undoubtedly the result of mesh selectivity. We observed no indication of sex reversal in the species.

Appendix Table 6 shows the length distributions in numbers of shrimp by sex for each cruise.

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APPENDIX TABLES

APPENDIX TABLE 1.—Mean median lengths of female *H. robustus* by depth, latitude, and cruise.

Item	322 to 366 m (176-200 fm)											
	Lat 29°00′ to 29°39′ N						Lat 29°40′ to 30°13′ N					
Cruise dates	Aug. 13-20 1957	Nov 20-25 1957	June 11-22 1958	Jan. 18-28 1960	Apr. 28-May 1 1961	Jan. 16-Feb. 22 1962	Aug. 13-20 1957	Nov. 20-25 1957	June 11-22 1958	Jan. 18-28 1960	Apr. 28-May 1 1961	Jan. 16-Feb. 22 1962
Number of stations	8	5	3	1	2	2	1	4	22	1	2	1
Mean median length (½ cm)	32.8	30.9	32.7	24.5	29.0	33.8	35.0	33.4	33.6	35.0	36.0	35.0

Item	368 to 411 m (201-225 fm)											
	Lat 29°00′ to 29°39′ N						Lat 29°40′ to 30°13′ N					
Cruise dates	Apr. 26-28 1957	July 17-30 1957	Aug. 13-20 1957	Aug. 22-28 1962	Feb 5-7 1964	Nov. 11-18 1964	Apr. 26-28 1957	July 17-30 1957	Aug. 13-20 1957	Aug. 22-28 1962	Feb 5-7 1964	Nov. 11-18 1964
Number of stations	2	2	4	2	4	15	1	1	2	2	1	5
Mean median length (½ cm)	25.8	30.0	31.9	30.0	29.6	25.4	38.0	38.0	35.3	36.0	37.0	27.0

APPENDIX TABLE 2.—Mean median lengths of female *H. robustus* by latitude, depth, and cruise.

Item	Lat 29°40′ to 30°13′ N											
	First depth 274-320 m (150-175 fm)				Second depth 322-366 m (176-200 fm)							
Cruise dates	May 29-31 1957	Jan. 18-28 1960	Apr. 28-May 1 1961	Jan. 16-Feb. 22 1962	May 29-31 1957	Jan. 18-28 1960	Apr. 28-May 1 1961	Jan. 16-Feb. 22 1962				
Number of stations	1	1	6	6	1	1	2	1				
Mean median length (½ cm)	37.0	34.0	34.9	35.6	37.0	35.0	36.0	35.0				
	First depth 322-366 m (176-200 fm)						Second depth 368-411 m (201-225 fm)					
Cruise dates	July 17-30 1957	Aug. 13-20 1957	Aug. 22-28 1962	Sept. 25-28 1962	Feb. 5-7 1964	Nov. 11-18 1964	July 17-30 1957	Aug. 13-20 1957	Aug. 22-28 1962	Sept. 25-28 1962	Feb. 5-7 1964	Nov. 11-18 1964
Number of stations	5	1	3	5	1	1	1	2	2	2	1	5
Mean median length (½ cm)	33.8	35.0	35.7	36.2	36.0	23.0	38.0	35.3	36.0	35.5	37.0	27.0
	First depth 368-411 m (201-225 fm)						Second depth 413 plus m (226 plus fm)					
Cruise dates	Sept. 25-28 1962						Sept. 25-28 1962					
Number of stations	2						1					
Mean median length (½ cm)	35.5						36.0					

Item	Lat 29°00′ to 29°39′ N									
	First depth 322-366 m (176-200 fm)					Second depth 368-411 m (201-225 fm)				
Cruise dates	April 26-28 1957	Aug. 13-20 1957	Nov. 20-25 1957	Jan. 18-28 1960	Apr. 30-May 3 1960	April 26-28 1957	Aug. 13-20 1957	Nov. 20-25 1957	Jan. 18-28 1960	Apr. 30-May 3 1960
Number of stations	2	8	5	1	6	2	4	2	1	4
Mean median length (½ cm)	30.0	32.8	30.9	24.5	31.3	25.8	31.9	29.0	33.0	30.6
	First depth 368-411 m (201-225 fm)					Second depth 413 plus m (226 plus fm)				
Cruise dates	July 17-30 1957		July 20-22 1967			July 17-30 1957		July 20-22 1967		
Number of stations	2		4			1		2		
Mean median length (½ cm)	30.0		25.3			29.0		27.8		

APPENDIX TABLE 3.—Regression of carapace length on total length for *H. robustus*.

Total length	Males	Females	Mean carapace length	
			Males	Females
mm	*Number*	*Number*	*mm*	*mm*
83	--	1	--	20.0
88	6	1	20.2	20.0
93	8	4	21.3	21.5
98	12	5	22.8	22.8
103	22	10	24.0	24.2
108	23	12	24.9	25.1
113	45	18	25.9	26.2
118	63	23	27.5	27.6
123	94	20	28.8	28.9
128	86	17	30.1	30.3
133	104	34	31.7	31.4
138	115	46	33.1	32.8
143	94	53	34.5	34.1
148	34	60	35.6	35.4
153	14	36	36.8	37.0
158	3	36	37.7	39.3
163	--	56	--	41.7
168	1	86	41.0	43.1
173	--	102	--	44.6
178	1	82	45.0	46.3
183	--	77	--	48.1
188	--	81	--	49.4
193	--	49	--	51.3
198	--	16	--	52.9
203	--	6	--	55.5
Total	725	931		

APPENDIX TABLE 4.—Length distributions of ovarian stages by season and cruise.
[UN = undeveloped; D = developing; P = pink; R = ripe]

Total length	Fall				Winter					
	Nov. 20-25, 1957		Nov. 11-18, 1964		Jan. 18-28, 1960		Jan. 16-Feb. 22, 1962		Feb. 5-7, 1964	
	UN + D	P + R	UN + D	P + R	UN + D	P + R	UN + D	P + R	UN + D	P + R
mm	%	%	%	%	%	%	%	%	%	%
53	--	--	--	--	--	--	--	--	--	--
58	--	--	0.2	--	--	--	--	--	--	--
63	--	--	0.2	--	--	--	--	--	--	--
68	--	--	0.7	--	--	--	--	--	--	--
73	--	--	0.9	--	--	--	0.1	--	0.3	--
78	0.1	--	1.1	--	0.3	--	0.1	--	0.3	--
83	0.5	--	1.2	--	2.4	--	0.1	--	0.7	--
88	0.5	--	1.1	--	3.4	--	0.3	--	0.7	--
93	--	--	1.0	--	6.7	--	0.3	--	1.6	--
98	0.7	--	1.2	--	5.3	--	0.2	--	3.3	--
103	2.3	--	2.7	--	8.0	--	0.6	--	5.8	--
108	5.2	--	6.1	--	2.4	0.3	0.7	--	1.3	--
113	5.6	--	9.0	--	1.5	--	0.3	--	2.0	--
118	5.2	--	12.7	--	1.2	--	1.3	0.1	2.6	--
123	3.2	--	13.7	0.2	1.8	--	1.8	0.1	3.6	--
128	2.7	0.1	12.2	0.1	3.2	--	1.0	0.1	6.1	--
133	2.4	0.6	7.3	0.1	2.4	--	1.0	0.1	4.3	--
138	1.5	1.6	4.0	0.1	2.8	0.9	0.5	0.1	2.6	0.7
143	2.1	2.8	2.7	0.4	0.3	0.6	0.3	0.1	2.3	1.0
148	1.0	2.7	2.7	0.9	0.9	1.8	0.7	0.3	1.0	0.3
153	0.8	3.2	2.5	1.4	1.5	3.0	0.5	0.9	1.0	1.6
158	1.5	3.1	1.4	1.0	2.4	3.3	1.4	1.4	1.0	2.3
163	0.4	4.5	1.2	1.1	1.5	4.2	1.9	4.2	0.3	2.0
168	--	7.4	0.7	0.7	0.6	7.5	2.7	8.5	0.7	3.0
173	--	8.5	0.6	1.2	1.2	3.9	4.4	12.4	0.7	3.6
178	0.4	6.5	0.3	0.8	1.5	5.8	3.7	12.9	1.6	4.6
183	0.5	9.6	0.5	0.5	0.9	4.1	1.8	12.9	1.6	8.5
188	0.1	7.7	0.4	0.3	2.1	3.6	1.4	8.4	3.0	6.9
193	0.3	2.6	0.2	1.6	1.2	2.7	1.0	5.2	3.0	8.2
198	--	1.6	0.2	0.6	0.6	0.3	0.4	2.1	1.0	3.0
203	--	0.5	--	0.2	0.6	0.9	0.1	1.0	--	1.6
208	--	--	--	--	0.3	0.3	0.1	0.5	--	0.3
213	--	--	--	0.1	--	--	--	--	--	--
218	--	--	--	--	--	--	--	--	--	--
Total	37.0	63.0	88.7	11.3	56.8	43.2	28.7	71.3	52.4	47.6
Number of females	278	476	1,071	136	193	146	392	975	160	145

APPENDIX TABLE 4.—Length distributions of ovarian stages by season and cruise—*Continued.*
[UN = undeveloped; D = developing; P = pink; R = ripe]

Total length	Spring									
	Apr. 26-28, 1957		May 29-31, 1957		June 11-22, 1958		Apr. 30-May 3, 1960		Apr. 28-May 1, 1961	
	UN + D	P + R	UN + D	P + R	UN + D	P + R	UN + D	P + R	UN + D	P + R
mm	%	%	%	%	%	%	%	%	%	%
53	--	--	--	--	--	--	--	--	--	--
58	--	--	0.3	--	--	--	--	--	--	--
63	0.2	--	--	--	--	--	--	--	--	--
68	--	--	--	--	--	--	--	--	--	--
73	0.2	--	--	--	--	--	--	--	0.2	--
78	0.3	--	0.5	--	--	--	--	--	0.2	--
83	1.6	--	0.3	--	--	--	0.2	--	0.3	--
88	1.3	--	--	--	--	--	--	--	0.5	--
93	1.8	--	--	--	0.1	--	0.9	--	2.5	--
98	3.8	--	0.3	--	0.2	--	0.4	--	2.4	--
103	4.2	--	0.3	--	0.4	--	2.0	--	3.2	--
108	2.6	--	0.5	--	0.2	--	4.2	--	2.5	--
113	3.0	--	1.1	--	0.9	--	5.3	--	2.5	0.2
118	2.4	--	0.8	--	1.3	--	5.5	0.2	1.5	--
123	3.2	0.2	1.1	--	2.2	--	4.6	--	0.5	--
128	5.9	0.2	1.6	--	2.9	--	2.4	--	0.8	--
133	7.8	0.3	3.2	--	4.6	--	4.0	--	0.5	--
138	6.4	0.5	2.9	--	5.7	0.1	5.7	0.7	2.0	0.2
143	4.2	1.2	3.2	0.8	4.6	0.9	3.1	1.3	1.7	0.4
148	2.2	0.8	1.1	1.6	3.4	1.6	2.9	1.6	1.2	1.2
153	0.6	0.5	1.1	1.1	4.0	2.5	2.4	2.0	1.5	2.7
158	1.1	0.8	0.3	0.5	3.8	2.8	3.1	2.0	1.5	2.6
163	2.2	1.2	1.3	3.5	3.1	3.8	3.3	4.0	1.0	3.5
168	2.2	1.6	1.1	7.2	3.2	3.0	3.5	4.0	1.5	7.2
173	2.2	3.0	1.3	7.5	3.3	3.9	3.5	5.0	2.4	10.4
178	1.9	2.5	1.3	8.8	3.6	4.4	4.4	4.7	1.0	12.5
183	3.4	2.4	1.6	12.7	3.7	5.5	2.2	2.4	1.0	8.5
188	3.7	4.0	1.9	11.3	4.1	4.1	2.2	1.6	1.2	6.7
193	4.2	2.3	1.3	6.7	2.8	3.4	1.5	0.9	0.7	5.1
198	2.6	1.0	--	6.2	1.7	2.6	0.2	1.1	--	2.2
203	0.8	0.5	0.5	2.4	0.4	0.7	0.4	0.2	0.2	1.2
208	0.6	0.2	--	0.8	0.2	0.1	0.2	0.2	--	0.5
213	0.2	--	--	--	--	0.1	--	--	--	0.2
218	--	--	--	--	--	0.1	--	--	--	--
Total	76.8	23.2	28.9	71.1	60.4	39.6	68.1	31.9	34.5	65.5
Number of females	480	144	108	265	1,098	713	310	144	205	384

APPENDIX TABLE 4.—Length distributions of ovarian stages by season and cruise—*Continued.*
[UN = undeveloped; D = developing; P = pink; R = ripe]

Total length	Summer									
	July 17-30, 1957		Aug. 13-20, 1957		Aug. 22-28, 1962		Sept. 25-28, 1962		July 20-22, 1967	
	UN + D	P + R	UN + D	P + R	UN + D	P + R	UN + D	P + R	UN + D	P + R
mm	%	%	%	%	%	%	%	%	%	%
53	--	--	--	--	--	--	--	--	--	--
58	--	--	--	--	--	--	--	--	--	--
63	--	--	--	--	--	--	--	--	--	--
68	--	--	--	--	--	--	--	--	--	--
73	--	--	--	--	--	--	--	--	0.3	--
78	--	--	0.3	--	--	--	0.3	--	--	--
83	0.2	--	0.2	--	0.2	--	0.3	--	--	--
88	0.2	--	0.5	--	--	--	--	--	--	--
93	0.9	--	0.9	--	0.8	--	--	--	0.7	--
98	1.3	--	1.9	--	0.6	--	0.8	--	1.3	--
103	2.2	--	3.3	--	0.6	--	0.8	--	7.4	--
108	2.6	--	3.9	0.1	3.3	--	1.6	--	10.1	--
113	4.0	--	3.8	--	4.8	--	0.8	--	13.4	--
118	4.9	--	3.3	--	3.5	--	0.8	--	13.7	--
123	2.9	--	2.8	--	3.3	--	1.3	--	8.1	--
128	1.5	--	1.3	--	3.5	--	1.1	--	3.7	--
133	4.4	--	3.2	--	2.9	--	0.5	--	3.7	--
138	5.4	0.2	4.4	0.2	1.7	--	1.6	--	5.4	0.3
143	4.8	0.4	4.0	0.2	1.3	--	0.8	--	1.0	0.3
148	3.9	0.4	4.3	0.8	1.7	--	0.8	--	2.4	--
153	2.9	0.8	2.3	0.4	1.0	0.2	2.1	--	3.4	0.7
158	1.1	0.2	3.2	0.5	1.7	0.2	1.3	--	2.4	1.3
163	3.1	0.4	6.2	0.8	6.5	0.4	3.6	1.1	1.3	0.3
168	3.1	2.0	6.0	1.8	9.1	1.0	6.4	1.3	2.7	2.0
173	4.2	4.6	7.2	2.1	10.7	1.5	9.9	1.9	2.7	1.3
178	3.5	3.8	6.5	1.2	14.7	1.9	18.3	1.9	1.0	3.4
183	3.3	5.1	5.5	2.3	10.7	1.0	14.8	2.4	1.0	1.7
188	3.3	7.5	4.3	2.4	6.3	--	9.9	0.8	0.7	1.3
193	2.6	5.0	2.8	1.1	2.5	0.4	6.4	1.3	0.7	--
198	0.9	1.3	1.1	0.9	0.8	--	2.9	0.8	--	0.3
203	0.2	0.9	1.1	0.5	1.0	--	1.1	--	--	--
208	--	--	0.3	0.1	0.2	--	--	--	--	--
213	--	--	--	--	--	--	0.3	--	--	--
218	--	--	--	--	--	--	--	--	--	--
Total	67.4	32.6	84.6	15.4	93.4	6.6	88.5	11.5	87.1	12.9
Number of females	305	149	738	136	487	34	330	43	259	39

APPENDIX TABLE 5.—Length distributions by sex for season and cruise.

Total length	Fall				Winter					
	Nov. 20-25, 1957		Nov. 11-18, 1964		Jan. 18-28, 1960		Jan. 16-Feb. 22, 1962		Feb. 5-7, 1964	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
mm	%	%	%	%	%	%	%	%	%	%
53	--	--	--	--	--	--	--	--	--	--
58	--	--	--	0.2	--	--	--	--	--	--
63	--	--	--	0.2	--	--	--	--	--	--
68	0.2	--	0.1	0.7	--	--	--	--	--	--
73	--	--	--	0.9	--	--	--	0.1	--	0.3
78	0.5	--	0.2	1.1	0.7	0.3	--	0.1	--	0.3
83	0.9	0.5	0.4	1.1	1.7	2.3	--	0.1	0.7	0.7
88	0.2	0.5	0.4	1.1	3.7	3.4	0.1	0.1	2.1	0.7
93	0.9	--	0.2	1.0	3.7	6.7	0.1	0.3	5.2	1.6
98	3.9	0.7	1.7	1.1	3.4	5.1	0.6	0.3	6.2	3.2
103	7.7	2.3	4.9	2.6	2.0	7.6	0.8	0.2	5.9	5.8
108	10.0	5.2	9.3	6.0	2.0	2.5	1.6	0.6	4.5	1.3
113	10.3	5.6	14.6	8.8	1.7	1.4	1.8	0.7	4.5	1.9
118	8.2	5.2	16.5	12.8	3.4	1.1	3.2	0.3	6.6	2.6
123	9.1	3.2	12.1	13.4	7.6	2.0	3.6	1.8	3.1	3.6
128	14.0	2.8	11.3	12.4	20.4	3.1	9.0	1.8	7.9	6.1
133	12.0	2.9	8.6	7.3	18.6	2.5	20.4	1.3	9.3	4.2
138	9.8	3.1	6.2	4.1	15.4	3.9	26.4	0.9	10.0	3.2
143	6.4	4.9	4.2	3.0	9.6	0.8	21.3	0.4	17.6	3.6
148	5.2	3.7	4.2	3.4	2.7	2.5	7.4	0.8	11.0	1.3
153	0.5	4.1	2.6	4.1	2.0	5.1	1.8	1.6	4.1	2.9
158	--	4.5	0.9	2.4	0.5	5.3	0.8	2.8	0.7	3.2
163	--	4.9	1.0	2.4	--	5.6	0.6	6.6	--	2.3
168	--	7.4	0.4	1.4	0.5	8.7	0.1	10.0	--	3.6
173	0.2	8.6	--	2.0	0.2	5.6	0.1	17.0	--	4.2
178	--	6.9	0.1	1.4	0.2	7.6	0.1	16.3	0.3	6.5
183	--	10.1	0.1	1.2	--	4.8	0.1	15.4	0.3	10.0
188	--	8.0	--	0.8	--	5.3	0.1	9.7	--	9.7
193	--	2.8	--	1.8	--	3.7	--	6.3	--	11.4
198	--	1.6	--	0.9	--	1.1	--	2.7	--	3.9
203	--	0.5	--	0.2	--	1.4	--	1.2	--	1.6
208	--	--	--	0.1	--	0.6	--	0.6	--	0.3
213	--	--	--	0.1	--	--	--	--	--	--
218	--	--	--	--	--	--	--	--	--	--
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Number of shrimp	441	755	863	1,233	408	356	881	1,386	290	309

APPENDIX TABLE 5.—Length distributions by sex for season and cruise.—*Continued.*

Total length	Spring									
	Apr. 26-28, 1957		May 29-31, 1957		June 11-22, 1958		Apr. 30-May 3, 1960		Apr. 28-May 1, 1961	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
mm	%	%	%	%	%	%	%	%	%	%
53	--	--	--	--	--	--	--	--	--	--
58	--	--	--	0.3	--	--	--	--	--	--
63	--	0.2	0.5	--	--	0.1	--	--	--	--
68	0.2	--	--	--	--	--	--	--	--	--
73	0.4	0.2	--	--	--	--	--	--	--	0.2
78	0.8	0.3	--	0.5	--	--	--	--	--	0.2
83	1.0	1.6	--	0.3	--	--	--	0.2	0.6	0.3
88	1.8	1.3	0.5	--	0.3	--	--	--	0.8	0.5
93	5.7	1.8	2.1	--	0.3	0.1	0.3	0.9	2.0	2.5
98	5.1	3.8	0.5	0.3	0.4	0.2	0.5	0.4	3.9	2.3
103	5.5	4.2	2.7	0.3	0.7	0.4	2.4	1.9	5.1	3.2
108	5.1	2.6	7.0	0.5	2.5	0.2	4.2	4.1	3.1	2.5
113	6.5	3.0	4.3	1.1	4.2	0.9	4.0	5.4	1.4	2.7
118	8.5	2.4	8.0	0.8	7.6	1.3	7.7	5.6	1.8	1.5
123	12.2	3.4	17.2	1.1	19.1	2.2	16.0	4.5	3.3	0.5
128	7.3	6.1	13.4	1.6	17.6	2.9	19.2	2.6	16.4	0.8
133	8.3	8.2	10.8	3.2	18.3	4.7	18.1	3.9	21.9	0.5
138	9.3	6.9	13.9	3.0	11.2	5.9	13.5	6.3	19.4	2.2
143	10.2	5.5	6.4	4.0	9.1	5.4	7.9	4.3	11.9	2.0
148	8.5	3.0	7.5	2.7	5.5	5.0	1.6	4.3	4.3	2.5
153	2.4	1.1	2.7	2.1	1.7	6.5	1.6	4.5	2.5	4.3
158	0.8	1.9	--	0.8	0.2	6.6	0.8	5.0	0.8	4.0
163	0.2	3.4	--	4.8	0.4	6.9	1.1	7.6	--	5.0
168	--	3.8	--	8.3	--	6.1	0.3	7.7	--	9.0
173	0.2	5.3	0.5	8.8	0.3	7.2	0.5	8.9	0.2	12.7
178	--	4.5	0.5	10.2	0.4	8.1	--	8.9	0.2	13.0
183	--	5.8	0.5	14.2	0.1	9.1	0.3	4.5	0.2	9.7
188	--	7.6	--	13.1	0.1	8.2	--	3.7	--	8.0
193	--	6.3	--	8.0	--	6.2	--	2.4	0.2	5.7
198	--	3.5	0.5	6.2	--	4.3	--	1.3	--	2.2
203	--	1.3	0.5	3.0	--	1.2	--	0.7	--	1.3
208	--	0.8	--	0.8	--	0.2	--	0.4	--	0.5
213	--	0.2	--	--	--	0.1	--	--	--	0.2
218	--	--	--	--	--	--	--	--	--	--
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Number of shrimp	495	624	187	373	1,136	1,811	379	463	489	601

APPENDIX TABLE 5.—Length distributions by sex for season and cruise.—*Continued.*

Total length	Summer									
	July 17-30, 1957		Aug. 13-20, 1957		Aug. 22-28, 1962		Sept. 25-28, 1962		July 20-22, 1967	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
mm	%	%	%	%	%	%	%	%	%	%
53	--	--	--	--	--	--	--	--	--	--
58	--	--	--	--	--	--	--	--	--	--
63	--	--	--	--	--	0.2	--	--	--	--
68	--	--	--	--	--	--	--	--	--	--
73	--	--	--	--	0.3	--	--	--	--	0.3
78	--	--	0.4	0.3	--	--	--	0.3	--	--
83	--	0.2	0.4	0.2	--	0.2	--	0.3	0.4	--
88	1.7	0.2	1.1	0.5	0.5	--	0.3	--	--	--
93	2.3	0.9	1.3	0.9	1.5	0.8	0.5	--	0.8	0.7
98	3.4	1.3	2.3	1.9	3.3	0.6	0.8	0.8	3.2	1.3
103	5.7	2.2	4.5	3.3	6.9	0.6	1.9	0.8	11.2	7.4
108	5.1	2.6	5.9	4.0	8.1	3.2	0.8	1.6	9.2	10.0
113	8.3	4.0	4.1	3.8	7.9	4.8	3.0	0.8	13.8	13.4
118	7.1	4.8	7.4	3.3	3.8	3.4	2.7	0.8	10.0	13.6
123	9.4	2.9	14.9	2.8	8.1	3.2	3.2	1.3	12.3	8.0
128	11.4	1.5	13.0	1.3	12.0	2.9	5.4	1.3	10.0	3.7
133	12.6	4.4	15.4	3.2	19.8	2.9	16.4	0.5	11.5	3.7
138	13.8	5.5	15.4	4.6	18.6	1.7	28.3	1.9	6.8	5.7
143	13.4	5.0	8.9	4.2	4.3	1.3	25.5	0.8	7.2	1.3
148	2.6	4.4	4.4	5.2	4.1	1.7	8.8	0.8	0.8	2.7
153	1.7	3.7	0.6	2.8	0.5	1.1	1.3	2.1	1.6	4.0
158	0.9	1.5	--	3.8	--	1.9	0.5	1.3	0.8	3.7
163	--	3.5	--	7.0	0.3	6.8	--	4.5	0.4	1.7
168	0.3	5.0	--	7.8	--	10.1	0.3	7.7	--	4.7
173	--	8.8	--	9.1	--	12.0	0.3	11.7	--	4.0
178	0.3	7.2	--	7.6	--	17.6	--	19.9	--	4.4
183	--	8.4	--	7.8	--	11.8	--	17.1	--	2.7
188	--	10.8	--	6.7	--	6.3	--	10.9	--	2.0
193	--	7.9	--	4.0	--	2.7	--	7.7	--	0.7
198	--	2.2	--	1.9	--	1.0	--	3.7	--	0.3
203	--	1.1	--	1.5	--	1.0	--	1.1	--	--
208	--	--	--	0.5	--	0.2	--	--	--	--
213	--	--	--	--	--	--	--	0.3	--	--
218	--	--	--	--	--	--	--	--	--	--
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Number of shrimp	350	456	798	874	393	525	373	376	251	299

APPENDIX TABLE 6.—Length distributions by cruise and sex.

Total length	Apr. 26-28, 1957		May 29-31, 1957		July 17-30, 1957		Aug. 13-20, 1957		Nov. 20-25, 1957	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
mm	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
53	--	--	--	--	--	--	--	--	--	--
58	--	--	--	1	--	--	--	--	--	--
63	--	1	1	--	--	--	--	--	--	--
68	1	--	--	--	--	--	--	--	1	--
73	2	1	--	--	--	--	--	--	--	--
78	4	2	--	2	--	--	3	3	2	--
83	5	10	--	1	--	1	3	2	4	4
88	9	8	1	--	6	1	9	4	1	4
93	28	11	4	--	8	4	10	8	4	--
98	25	24	1	1	12	6	18	17	17	5
103	27	26	5	1	20	10	36	29	34	17
108	25	16	13	2	18	12	47	35	44	39
113	32	19	8	4	29	18	33	33	46	42
118	42	15	15	3	25	22	59	29	36	39
123	61	21	32	4	33	13	119	24	40	24
128	36	38	25	6	40	7	104	11	62	21
133	41	51	20	12	44	20	123	28	53	22
138	46	43	26	11	48	25	123	40	43	23
143	51	34	12	15	47	23	71	37	28	37
148	42	19	14	10	9	20	35	45	23	28
153	12	7	5	8	6	17	5	24	2	31
158	4	12	--	3	3	7	--	33	--	34
163	1	21	--	18	--	16	--	61	--	37
168	--	24	--	31	1	23	--	68	--	56
173	1	33	1	33	--	40	--	80	1	65
178	--	28	1	38	1	33	--	67	--	52
183	--	36	1	53	--	38	--	68	--	77
188	--	48	--	49	--	49	--	59	--	61
193	--	40	--	30	--	36	--	35	--	21
198	--	22	1	23	--	10	--	17	--	12
203	--	8	1	11	--	5	--	13	--	4
208	--	5	--	3	--	--	--	4	--	--
213	--	1	--	--	--	--	--	--	--	--
218	--	--	--	--	--	--	--	--	--	--
Total	495	624	187	373	350	456	798	874	441	755
Number of stations	7		3		9		18		14	

APPENDIX TABLE 6.—Length distributions by cruise and sex.—*Continued.*

Total length	Aug. 22-28, 1962		Sept. 25-28, 1962		Feb. 5-7, 1964		Nov. 11-18, 1964		July 20-22, 1967	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
mm	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
53	--	--	--	--	--	--	--	--	--	--
58	--	--	--	--	--	--	--	2	--	--
63	--	1	--	--	--	--	--	2	--	--
68	--	--	--	--	--	--	1	8	--	--
73	1	--	--	--	--	1	--	11	--	1
78	--	--	--	1	--	1	2	13	--	--
83	--	1	--	1	2	2	3	14	1	--
88	2	--	1	--	6	2	3	13	--	--
93	6	4	2	--	15	5	2	12	2	2
98	13	3	3	3	18	10	15	14	8	4
103	27	3	7	3	17	18	42	32	28	22
108	32	17	3	6	13	4	80	74	23	30
113	31	25	11	3	13	6	127	110	35	40
118	15	18	10	3	19	8	143	159	25	41
123	32	17	12	5	9	11	105	167	31	24
128	47	15	20	5	23	19	97	154	25	11
133	78	15	61	2	27	13	74	90	29	11
138	73	9	100	7	29	10	53	50	17	17
143	17	7	95	3	51	11	36	37	18	4
148	16	9	33	3	32	4	36	42	2	8
153	2	6	5	8	12	9	22	50	4	12
158	--	10	2	5	2	10	8	29	2	11
163	1	36	--	17	--	7	9	29	1	5
168	--	53	1	29	--	11	3	17	--	14
173	--	63	1	44	--	13	--	25	--	12
178	--	93	--	75	1	20	1	17	--	13
183	--	62	--	64	1	31	1	15	--	8
188	--	33	--	41	--	30	--	10	--	6
193	--	14	--	29	--	35	--	22	--	2
198	--	5	--	14	--	12	--	11	--	1
203	--	5	--	4	--	5	--	2	--	--
208	--	1	--	--	--	1	--	1	--	--
213	--	--	--	1	--	--	--	1	--	--
218	--	--	--	--	--	--	--	--	--	--
Total	393	525	373	376	290	309	863	1,233	251	299
Number of stations	10		8		6		21		6	

APPENDIX TABLE 6.—Length distributions by cruise and sex.—*Continued.*

Total length	June 11-22, 1958		Jan. 18-28, 1960		Apr. 30-May 3, 1960		Apr. 28-May 1, 1961		Jan. 16-Feb. 22, 1962	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
mm	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
53	--	--	--	--	--	--	--	--	--	--
58	--	--	--	--	--	--	--	--	--	--
63	--	1	--	--	--	--	--	--	--	--
68	--	--	--	--	--	--	--	--	--	--
73	--	--	--	--	--	--	--	1	--	1
78	--	--	3	1	--	--	--	1	--	1
83	--	--	7	8	--	1	3	2	--	2
88	3	--	15	12	--	--	4	3	1	2
93	3	2	15	24	1	4	10	15	1	4
98	4	3	14	18	2	2	19	14	5	4
103	8	7	8	27	9	9	25	19	7	3
108	28	3	8	9	16	19	15	15	14	8
113	48	17	7	5	15	25	7	16	16	10
118	86	23	14	4	29	26	9	9	28	4
123	218	40	31	7	61	21	16	3	32	25
128	201	53	83	11	73	12	80	5	79	25
133	209	85	76	9	69	18	107	3	180	18
138	128	107	63	14	51	29	95	13	232	13
143	104	98	39	3	30	20	58	12	188	5
148	62	91	11	9	6	20	21	15	65	11
153	19	117	8	18	6	21	12	26	16	22
158	2	119	2	19	3	23	4	24	7	39
163	4	125	--	20	4	35	--	30	5	91
168	--	111	2	31	1	36	--	54	1	138
173	3	130	1	20	2	41	1	77	1	236
178	4	146	1	27	--	41	1	79	1	225
183	1	166	--	17	1	21	1	58	1	213
188	1	149	--	19	--	17	--	48	1	135
193	--	113	--	13	--	11	1	34	--	88
198	--	78	--	4	--	6	--	13	--	38
203	--	22	--	5	--	3	--	8	--	17
208	--	4	--	2	--	2	--	3	--	8
213	--	1	--	--	--	--	--	1	--	--
218	--	--	--	--	--	--	--	--	--	--
Total	1,136	1,811	408	356	379	463	489	601	881	1,386
Number of stations	28		13		10		12		24	

SEX PHEROMONE ACTIVITY OF THE MOLTING HORMONE, CRUSTECDYSONE, ON MALE CRABS

(*Pachygrapsus crassipes*, *Cancer antennarius*, AND *C. anthonyi*)

JAMES S. KITTREDGE,[1] MICHELLE TERRY,[1] AND FRANCIS T. TAKAHASHI[2]

ABSTRACT

The pheromone released by premolt female *Pachygrapsus crassipes* is a heat stable non-ionic polar lipid. The coincidence of the release of the pheromone and the nubial molt suggested that the molting hormone, crustecdysone, may also function as a sex pheromone. Adult male crabs were observed to display typical precopulatory behavior when exposed to dilute solutions of crustecdysone. The threshold concentration for behavioral response was found to be 10^{-13} M for *P. crassipes*, 10^{-10} M for *Cancer antennarius* and 10^{-8} M for *C. anthonyi*. These findings provide the basis for a theory of the evolution of pheromone communication in the Arthropoda.

The dominant position of chemoreception in the behavior of marine invertebrates and the implication of sex pheromones in the reproductive activities of many species is supported by many behavioral observations, but no pheromone has yet been characterized from the marine environment. In many marine decapod Crustacea copulation takes place immediately after the female molts. The male of the species recognizes the premolt condition of the female, is attracted to her, and usually seizes and carries her until she molts. This recognition at a distance has been reported for many genera of Crustacea (Hay, 1905; L. Agassiz in Verrill, 1908; Needler, 1931; Burkenroad, 1947; Williamson, 1953; Hughes and Matthiessen, 1962; Knudsen, 1964; Snow and Neilsen, 1966). Ryan (1966) described the search and display behavior exhibited by male *Portunus anguinotentus* when a premolt female crab was placed in the holding tank with them. Each male became active, walked about on the tips of its dactyls, elevated its body, and extended its chelae. When thus stimulated they often attempted to pull any crab they met into a precopulatory carrying position. Ryan demonstrated that this behavior is released by a sex pheromone in the urine of the premolt female crab.

METHODS

BIOASSAY

Observation vessels for determining the response of male *Pachygrapsus crassipes* to dilute solutions of molting hormone were constructed from 4-liter beakers. With a glass blowing torch and the edge of a carbon flat we formed an indent in the side of each beaker approximately 4 cm deep, parallel to and 3 cm above the bottom of the beaker. The outside of the beakers was masked with black paint with the exception of an 8 cm window opposite the indent. When a crab was placed in seawater in the observation chamber, they always scurried into the niche between the bottom of the beaker and the indent. If the seawater contained molting hormone, the crabs were stimulated to come out of the niche and assume a premating stance. The time elapsing after adding a solution of crustecdysone in seawater to an empty vessel containing a male crab until the crab elevated its cephalothorax in a typical stance was noted. Six crabs were timed at each concentration of

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crusteedysone, and fresh male crabs were used for each different concentration. The male crabs were held for several days in isolation from female crabs before testing.

ISOLATION COLUMNS

Columns (5 × 50 cm) of Amberlite XAD-2, a divinylbenzene polymer, were found to be effective in the recovery of polar steroids from seawater. The columns were washed with three void volumes of water to remove the salts, and the polar steroids were eluted with three volumes of 60% ethanol. The more nonpolar lipids were removed with 95% ethanol. After repeated use the columns were reconditioned by cycling through 95% ethanol, diethyl ether, hexane, diethyl ether, ethanol, and water.

FRACTIONATION COLUMNS

Chromosorb 102, which is an 80/100 mesh fraction of Amberlite XAD-2, in a 0.9 × 100 cm column was used to fractionate the polar lipids. This column was eluted with a gradient of ethanol (20% to 80%) (Hori, 1969). The gradient was formed by an Isco Dialagrad dual pump[3] with the following settings: 40 ml hr; reservoir for "A" pump, 80% ethanol; reservoir for "B" pump, 20% ethanol; percentage settings for "B" pump, 100, 100, 90, 80, 70, 60, 50, 30, 15, 0, 0 (this pump makes five intermediate linear steps between each setting); total time 16 hr. The fractionation was monitored at 254 μ with an Isco Model UA-2 UV monitor and fractions were collected in an Isco Model 327 fraction collector.

A silicic acid column (0.6 × 30 cm) eluted with chloroform-ethanol (5:1, v/v) and monitored in the UV was employed for further fractionation (Horn et al., 1968).

OBSERVATIONS ON THE PREMATING BEHAVIOR AND THE SEX PHEROMONE OF *Pachygrapsus crassipes*

Although the premating behavior of the lined shore crab, *Pachygrapsus crassipes*, as described by others (Hiatt, 1948; Bovbjerg, 1960), did not include the typical stance of other Brachyura, the abundance and ease of collection of this species prompted us to re-examine their behavior. We found that male *P. crassipes*, in the presence of a premolt female, exhibit an easily recognizable stance. The males elevate their cephalothoraxes and tilt the anterior margin up. They walk on the tips of the dactyls of their first three pairs of walking legs and extend their fourth pair horizontally backwards. The chelipeds are partially extended but lowered, as opposed to the elevated defensive position. When thus stimulated they will often attempt to seize any other *P. crassipes* they encounter, male or female, and turn them over into the holding position with which they maintain control of a premolt female. This behavior compares with that described by Ryan (1966) for male *P. sanguinolentus*. There were two additional characteristics of the male *P. crassipes* behavior that paralleled the premating behavior of *Cancer magister* as described by Snow and Neilsen (1966). They observed that the male *C. magister*, while carrying the female, frequently extended his fourth pair of walking legs straight back. We have observed that *C. magister* will thus extend his legs while holding his body elevated when stimulated by the sex pheromone before he seizes the female, as does *P. crassipes*. Snow and Neilsen (1966) also noted that "on occasion the male would rise up on the tips of his walking legs and raise the female up into an elevated position nearly 6 inches off the bottom of the tank. This movement would be accompanied by a continuous flexing of the male's abdominal flap." A frequent observation with pairs of *P. crassipes* was that they would stand facing each other, both with their legs extended and body elevated, but with the male higher. In this position the female would lower her abdominal flap slowly and then flex it rapidly but not into a completely retracted position. She would repeat this movement several times. The male would then repeat an identical movement of his abdominal flap.

[3] Reference to commercial products does not imply endorsement.

This aspect of their behavior could be interpreted as a courting gesture, but in the context of chemical communication we prefer to interpret this as a fanning motion facilitating the distribution of pheromones that may be aphrodisiac in nature. Commercial fishermen for both the American lobster, *Homarus americanus*, and the California spiny lobster, *Panulirus interruptus*, have suspected that the males of each species could be used to attract the females.

A single active premolt female *Pachygrapsus crassipes* released sufficient pheromone to stimulate all of the males in a 25-gal recirculating aquarium. A single female *P. crassipes* in an aerated 2-gal container released a pheromone which stimulated a male *Cancer antennarius* to exhibit a premating stance.

With these observations providing the bioassay, we examined the nature of the sex pheromone. Water which had contained a premolt female crab was boiled for 10 min, cooled, and aerated. The active principle was still present. The active principle was not retained by cation nor anion exchange resins nor by charcoal. The active substance could, however, be extracted from "active seawater" with isopropanol/diethyl ether. These observations, together with the premolt condition of the active females, led us to suspect that the females might be releasing molting hormone into the water, and that this steroid might be functioning as a sex pheromone.

BIOASSAY OF CRUSTECDYSONE

We soon confirmed that dilute solutions of crustecdysone (β-ecdysone, 20R-hydroxyecdysone, ecdysterone, isoinokosterone), which is one of the molting hormones of Crustacea (Horn et al., 1968), elicited a typical response from male *P. crassipes*. In order to establish the threshold concentration that would release response we standardized the conditions for the bioassay. As described under "Methods," the test adopted allowed the male crab to be flooded with a known concentration of the steroid in seawater, in contrast to the diffusion techniques often employed. *P. crassipes* proves to be ideal for this mode of testing because they are an intertidal species and in nature normally leave the tide pools at low tide to feed on the rocks. They were not disturbed on being placed in a wet empty observation vessel and sought out the artificial niche provided. On flooding with seawater they would remain in the niche for long periods or occasionally come out to explore briefly and then return to the niche. When a male *P. crassipes* was flooded with a solution of crustecdysone in seawater, he was stimulated to come out of the niche and explore the vessel and then to assume the premating stance. The time elapsing until the crab raised its body to assume the stance was found to be a function of the concentration of crustecdysone. Although the male crabs would often exhibit a full stance in a brightly illuminated laboratory at the higher concentrations of crustecdysone, the response was often erratic. All of the bioassays were conducted in an isolated room illuminated with an Eastman darkroom lamp with a 15-w bulb and an Eastman No. 00 yellow filter. The observer was stationed quietly before the "window" of the test vessel. On removal from the test vessel the male crabs were transferred to a 2-liter beaker of seawater to rinse off the extraneous crustecdysone and then were transferred to a small aquarium for further observation. In spite of the handling during the two transfers, male crabs that had been stimulated to display a premating stance in the observation vessel usually resumed this posture shortly after being transferred to the aquarium. When thus stimulated they often attempted to seize any other male crab in the aquarium.

All of the male *P. crassipes* utilized in establishing the response curve were collected at the same time and in the same area where we had just succeeded in collecting a number of premolt females. They were all held for three or more days in isolation from any female crabs. The curve was started with a concentration of 10^{-5} M crustecdysone in filtered seawater. At this concentration the response was rapid, averaging 7 sec. Succeeding tests were performed with ten-fold dilutions of the crustecdysone. Fresh solutions of crustecdysone were prepared after three steps of dilution or at the start of each day's testing. Previous experience had

demonstrated that dilute solutions in seawater lost some or all of their activity on storage even at 0° C, presumably through bacterial degradation or adsorption. It was planned that six male crabs would be tested at each concentration and that the five most consistent times would be averaged; however, the response was found to be remarkably uniform and in all but three cases all six crabs responded within a narrow time range. There was no sharp threshold of concentration. The average response times plotted as a smooth curve extending to 10^{-13} M crustecdysone concentration where the average response time was 22 min (Figure 1). No response was observed at 10^{-14} M. The scatter of response times was greatest at 10^{-5} M, probably because of the short-term disturbance of the male crab during flooding. The standard deviation of the normalized response times for 15 crabs, from 10^{-6} M to 10^{-13} M crustecdysone, was 8.6.

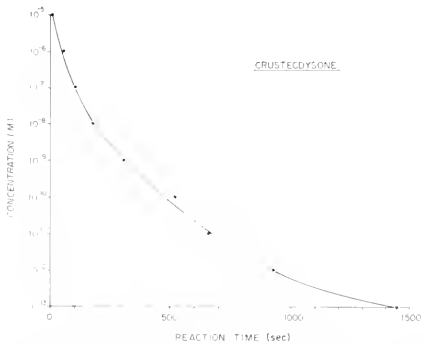

FIGURE 1.—Time elapsed following immersion of male *Pachygrapsus crassipes* in seawater solutions of crustecdysone before the body elevation phase of the precopulatory behavior.

Since the precopulatory behavior of males in the presence of premolt females appears to be general among the Brachyura, we examined the response of two species of *Cancer* to crustecdysone. Both *C. antennarius* and *C. anthonyi* displayed typical premating behavior when exposed to dilute solutions of crustecdysone. When the response time vs. concentration study was carried out with these two species each yielded a response curve similar to that developed by *P. crassipes*. There was, however, a marked difference. There was an abrupt break in the response yielding a distinct threshold at 10^{-10} M for *C. antennarius* and 10^{-8} M for *C. anthonyi*.

We then attempted to determine if *C. antennarius* males could detect a gradient in the concentration of crustecdysone. For this purpose we employed a simple "T" maze with a baffle at the head of each arm separating the seawater sources and forming mixing chambers. Five male *C. antennarius* were placed at the head of the maze and the water flows from each arm balanced. While seawater alone was flowing through the maze the *C. antennarius* remained quiet in the two corners at the origin of the maze. When a flow of crustecdysone solution was added to the mixing chamber at the head of one arm all five of the male *C. antennarius* soon became active. They explored up and down each arm of the maze but demonstrated no tendency to select the arm containing the crustecdysone. While these tests did not indicate any ability to detect or follow up a gradient, there was a positive response to the crustecdysone. All five of the male crabs were stimulated simultaneously to undertake an active exploratory behavior when the crustecdysone was introduced.

DEVELOPMENT OF THE TECHNIQUES FOR THE ISOLATION AND FRACTIONATION OF THE SEX PHEROMONE(S)

Liquid-liquid extraction procedures are inefficient for the recovery of trace quantities of polar lipids. Columns of Amberlite XAD-2 have been employed for the recovery of steroids from urine (Bradlow, 1968; Shackleton, Sjövall, and Wisén, 1970). Recently, Hori (1969) has employed a column of this resin eluted with a li-

near gradient of ethanol in water for the fractionation of the phytoecdysones. We have found that a column of XAD-2 could be used to recover traces of crustecdysone from seawater and from crab urine. Using the above two columns we have examined the seawater in which female *P. crassipes*, *C. magister*, and *C. productus* had been maintained for 3 to 6 hr. The product of individual *Cancer* were assayed, while the seawater from two or more *P. crassipes* was combined before extraction. We have also examined the urine of female *C. magister*. The *Cancer* were staged according to Drach (1939), and the female *P. crassipes* were selected for activity by observing the behavior of males in their presence. During our observations we found the female *C. magister* continued to release a pheromone for up to 2 weeks post molt.

The material recovered from the isolation column in 60% ethanol was reduced in volume to a few microliters and transferred in 20% ethanol to the Chromosorb 102 column. Elution of this column yielded an ultraviolet-absorbing peak near the front and two or more succeeding peaks. Each of three stage "D" *C. magister* and one stage "D" *C. productus* studied were found to have released an ultraviolet-absorbing compound that eluted from the column at the same ethanol concentration that a crustecdysone standard did. Both extracts of *P. crassipes* seawater yielded a peak in the position of crustecdysone. One stage "A" *C. magister* also yielded a peak in this position. A stage "C-4" *C. magister* did not yield any peak in the position of crustecdysone nor did urine from a stage "C-4" *C. magister* yield a peak in this position. Also one of the *C. magister* females that had yielded material eluting as crustecdysone while in stage "D" did not yield this substance during molting (stage "E"). The ultraviolet-absorbing fractions corresponding to crustecdysone from the above columns did not have an ultraviolet-absorption spectrum corresponding to that of crustecdysone. The absorption peak included the spectrum of crustecdysone, but had a double peak at a lower wave length. These fractions were concentrated and applied to a silicic acid column. The elution of this column with chloroform-ethanol yielded an ultraviolet-absorbing peak near the front and a peak eluting in the same volume as a crustecdysone standard. The material from this column has an ultraviolet-absorption spectrum that corresponds closely to that of crustecdysone.

We are now developing a derivitization technique that will permit us to subject our final samples to gas chromatography-mass spectrophotometry for structural conformation. Katz and Lensky (1970) have published a technique for the silylation of α-ecdysone for GLC analysis. We have employed silylation techniques with crustecdysone and observed decomposition during GLC.

RESULTS AND CONCLUSIONS

The pheromone released by *P. crassipes* stimulates premating behavior in *C. antennarius*. Male *C. magister* are excited into seizing and clasping female *C. productus* by their pheromone. Crustecdysone mimics the pheromone in its effects on male *P. crassipes*, *C. antennarius*, and *C. anthonyi* in the release of the premating stance. After exposure to crustecdysone all these species of male crabs attempt to seize other crabs, male or female, and pull them into a precopulatory position. In addition crustecdysone triggers a search behavior in male *C. antennarius*. These observations demonstrate a lack of specificity in the sex pheromones of these species and either that crustecdysone is the sex pheromone or sufficiently similar in molecular structure to the natural pheromones to mimic them.

A possible explanation of the discrepency between our results and those of Ryan (1966) that indicated a species specificity for the sex pheromones in the three species of crabs that he studied may be that some species may respond to deoxycrustecdysone, callinecdysone A (inokosterone) or callinecdysone B (makisterone), other ecdysones that have been isolated from Crustacea, (Gailbraith et al., 1968; Faux et al., 1969), or they may respond to one of the metabolic products of crustecdysone detected in insects (Gailbraith et al., 1969; Moriyama et al.,

1970; Cherbas and Cherbas, 1970; Heinrich and Hoffmeister, 1970).

The isolation and analysis of the material released into seawater by active female *P. crassipes*, *C. magister*, and *C. productus* demonstrated that a compound could be detected that is eluted from two different columns in the same position as crustecdysone and has a UV absorption spectra that is similar to that of crustecdysone.

The semilog plot of the response times for male *P. crassipes* to varying concentrations of crustecdysone is approximately parabolic, and the scatter of response times at each concentration is remarkably narrow. This, the range of response times, and the continued response of the male crabs after removal from the stimulus, permit an interpretation of the chemoreception of pheromones from dilute solutions. The observations suggest that the pheromone has a high affinity for the receptor site resulting in a long half life for the receptor-pheromone complex. Indeed, one might have postulated that even a polar steroid might be strongly bound to a lipoid receptor in an aqueous medium. It is apparent that the crabs are capable of summating the chemical information for a considerable period of time before a threshold which releases response behavior is reached. Though summation may take place at any level in the nervous system, the simplest interpretation suggests that this takes place at the receptors. This summation of "information quanta" can function either in extremely dilute solutions or, in nature, it would permit the accumulation of subthreshold amounts presented in random turbulences of the current from the source.

This finding has significance in a consideration of the evolution of pheromone communication. It has been suggested that chemical signals between cells were evolved before the evolution of the metazoans and that these signals were later internalized as hormones and synaptic transmitters (Haldane, 1955; Wilson, 1968). In the present instance we have a reversal of this internalization. The Crustacea, having evolved polar steroid hormones to regulate molting, on externalization of the receptor site onto chemoreceptor organs and on alteration of the resorption process in the antennual gland during the premolt stage of the females were then capable of signaling the approach of the nubial molt. This interpretation obviates the concern over the improbability of the simultaneous *de novo* origin of both the genetic information directing the biosynthesis of the pheromone and that concerned with the architecture of the receptor site. We may assume that an unmasking of that portion of the chromosome that specifies the receptor site for the hormone on the membranes of the target organs occurred in the chemosensory neurons. A masking of the active transport system for the hormone from the fluid of the antennule gland of the female is also assumed. These two innovations are reasonably small evolutionary steps and conceptually preferable to the two *de nova* origins that must be assumed otherwise. This evolutionary step, the pheromone function of a hormone, may have been the origin of pheromone communication in the Arthropoda, for once fixed because of its reproductive value, it was then susceptible to a gradual evolutionary drift toward a variety of more specific pheromones.

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CHARACTERISTICS OF SEA-SURFACE TEMPERATURE ANOMALIES

L. E. Eber[1]

ABSTRACT

Sea-surface temperature anomalies in the North Pacific Ocean, constructed from a 14-year series (1949-62) of monthly mean charts, exhibit numerous instances of quasi-stationary behavior. Selected examples from this series reveal a recurring pattern in which the principal feature is a positive or negative cell in the anomaly field, located approximately between lat 30° N and 50° N. The cell in this pattern is partially encircled by anomalies of opposite sign to the north, east, and south in a zone contiguous with the North American coast. This anomaly configuration, viewed with consideration of the associated sea-temperature field, suggests the existence of a standing wave in the current structure. Such a wave could affect the partitioning of the West Wind Drift Current as it approaches the coast and splits into northward and southward flowing branches. Physical data for verification of a standing wave are not available, but dimensional attributes inferred from the sea-temperature anomaly structure conform loosely to theoretical constraints.

The temperatures in the upper mixed layer of the ocean undergo annual cycles, induced by seasonal heating and cooling, which vary from year to year. This variation can be expressed in terms of departures from the mean annual temperature cycle obtained by averaging over a number of years. The magnitudes, areal distributions, and time changes of such departures define anomalous conditions in the surface layer of the sea.

The 14-year series, 1949-62, of monthly sea-surface temperature charts, published by Eber, Saur, and Sette (1968), provides a base for studying temperature variations in the North Pacific Ocean. Monthly anomaly charts were constructed from this series by taking the difference between the sea-surface temperature fields for each month and year and the corresponding monthly normal fields. The latter were obtained by computing the 14-year averages, by month, at grid points. A number of selected examples are presented here to show the character of some of the prominent and long-lasting anomalies that occurred in the North Pacific Ocean between 1949 and 1962.

Many of the features to be discussed are in the vicinity of the transition zone between the subarctic and subtropic oceanographic regions as described by Tully (1964). Through this zone, which is located approximately at lat 35° N to 45° N between long 160° E and 140° W, the surface current flows eastward as the West Wind Drift. The mean surface temperature distribution in this region is essentially zonal with isotherms oriented along the circles of latitude. The chart of the 14-year average for October (Figure 1) illustrates these characteristics. As the West Wind Drift approaches

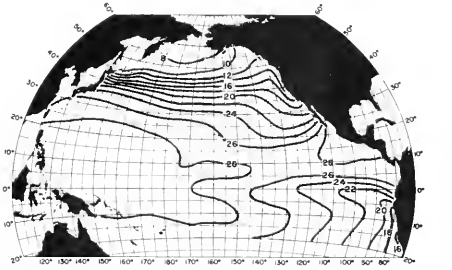

FIGURE 1.—Average sea-surface temperatures of the North Pacific Ocean in October, based on data from the 14-year period 1949-62.

the North American coast, it splits. One part turns north and moves in a counterclockwise trajectory around the Alaska Gyre and the other part turns south to become the California

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Current. The sea-surface isotherms, correspondingly, bend north and south, effecting a much-reduced temperature gradient parallel to the coast.

BEHAVIOR OF SEA-SURFACE TEMPERATURE ANOMALIES

To facilitate description, I shall designate as "warm" or "cold" cells, areas where the magnitude of departure from normal was 1° C or greater. The evolution of an anomaly can be readily followed by noting the configuration of its principal cell, or cells, in successive months. This is evident in the figures used to illustrate selected examples (Figures 2-30). These show

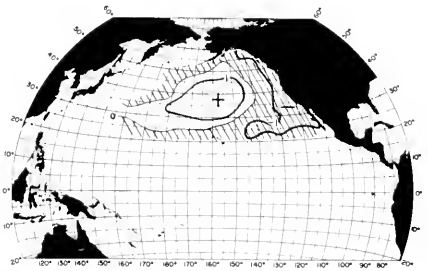

FIGURE 2.—Sea-surface temperature anomaly for January 1949. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (—) cells.

the sizes and locations of the principal cells, enclosed by heavy lines representing a magnitude of 1° C, in the regions relevant to the discussion. The cells are marked by plus or minus symbols according to the sign of the anomalies. The thin lines represent the zero anomaly contour separating areas of below normal temperature (hatched areas) from those where the temperature was above normal.

A good example of persistence was the warm anomaly present in the eastern North Pacific throughout most of 1949. In January the warm cell (defined by the 1° C anomaly contour) covered most of the area from lat 30° N to 45° N and long 145° W to 180° W. Maximum intensity of the positive departures from normal within this cell exceeded 3° C. Off the North American coast, the temperature anomalies were negative, with magnitudes greater than 1° C in a broad zone from Alaska to the tip of Baja California.

Figures 3-5 show subsequent positions of the warm cell in March, July, and October, respectively. The maximum intensities waxed and waned over this time period, dropping in March, increasing again to more than 3° C in July and diminishing once more in October. Except for a slight northward shift, the warm cell remained essentially stationary. During most of the period, negative anomalies prevailed in the coastal

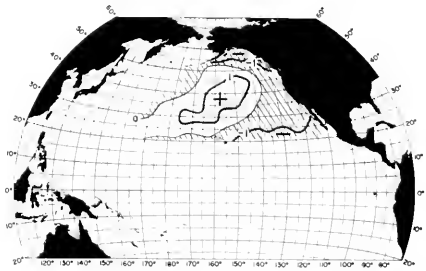

FIGURE 3.—Sea-surface temperature anomaly for March 1949. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (—) cells.

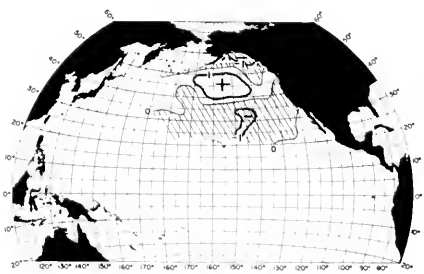

FIGURE 4.—Sea-surface temperature anomaly for July 1949. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (—) cells.

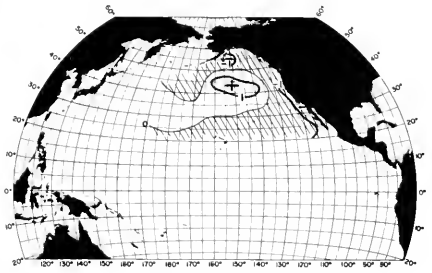

FIGURE 5.—Sea-surface temperature anomaly for October 1949. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (−) cells.

zone and in a southwestward tongue below 30° N but with fluctuating magnitudes.

The warm cell shrank in November and lost its specific identity in December. However, the general configuration of the colder than normal coastal zone and warmer than normal offshore region persisted through the winter and spring of 1950. In May 1950, the positive anomalies intensified and spread westward to Japan. The pattern stabilized in June and July marked by prominent warm cells in the east and far west portions of the positive area (Figure 6). At the same time, a cold cell in the Gulf of Alaska had begun to grow and push southward; this

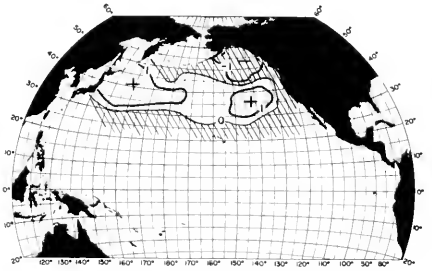

FIGURE 6.—Sea-surface temperature anomaly for July 1950. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (−) cells.

movement continued through September (Figure 7) accompanied by an eastward expansion of the warm belt. Ultimately, there was a deformation of the broad-scale pattern which had for many months dominated the central and eastern North Pacific, as negative anomalies spread through the Gulf of Alaska.

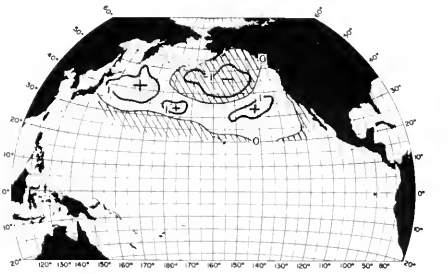

FIGURE 7.—Sea-surface temperature anomaly for September 1950. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (−) cells.

The years 1951-54 presented no striking examples of persistent sea temperature anomalies. Some relatively large-scale anomalies did develop in this period but they were short-lived. Much of the time the anomaly field was flat and featureless. Midway through 1955, however, a long-term progression of events began to evolve with the development of an anomaly pattern which was characterized by a positive belt that stretched eastward from Japan and a negative zone along most of the North American coast. The anomaly field intensified in August (Figure 8) when maxima exceeded 2° C in the warm cell and an extensive cold area was present in the coastal zone with negative departures exceeding 1° C in magnitude. The negative area stretched southwestward below lat 30° N. This pattern prevailed through November 1955 (Figure 9) but became weaker in December. In January 1956, the positive area contracted to the west while the region of negative anomalies to the east became flat and disorganized. Figure 10 shows the situation in February 1956. The

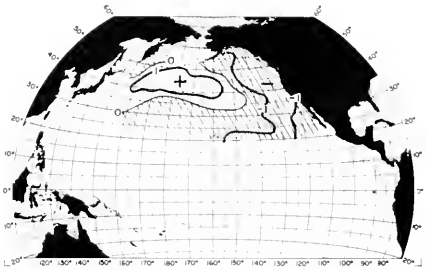

FIGURE 8.—Sea-surface temperature anomaly for August 1955. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (−) cells.

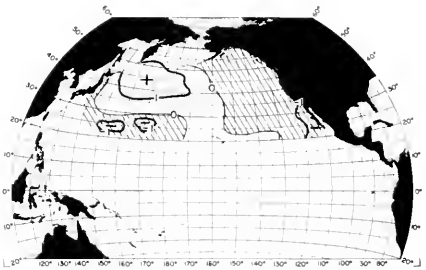

FIGURE 10.—Sea-surface temperature anomaly for February 1956. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (−) cells.

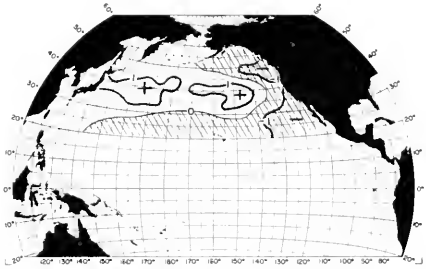

FIGURE 9.—Sea-surface temperature anomaly for November 1955. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (−) cells.

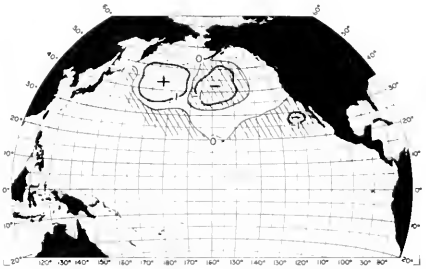

FIGURE 11.—Sea-surface temperature anomaly for August 1956. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (−) cells.

warm cell at lat 36° N to 50° N, long 175° W to 150° E was the only prominent feature in evidence and remained so until August 1956, when a large cold cell developed just east of it (Figure 11). This latter feature was short-lived, however, and during the next few months the positive area once again expanded eastward while the tendency of anomalies along the North American coast changed from variable to predominantly negative. The chart for November 1956 (Figure 12) depicts the result of this process.

Transition to a second phase in the long-term evolution of the anomaly field was foreshadowed by a small cold cell at lat 25° N to 30° N, long 160° E to 170° E (Figure 12). This cold cell grew in size but remained in about the same location through March 1957 (Figure 13) while the area of positive anomalies edged toward the North American coast. The transition was complete by June, when the cold cell shifted north and stretched eastward in a belt of negative anomalies between lat 30° N and 40° N (Figure 14). Positive values prevailed in the Gulf of Alaska and along the North American coast, effecting an almost complete reversal from the late 1955 pattern.

The negative anomalies in the west central sector, long 175° W to 160° E, contracted to form

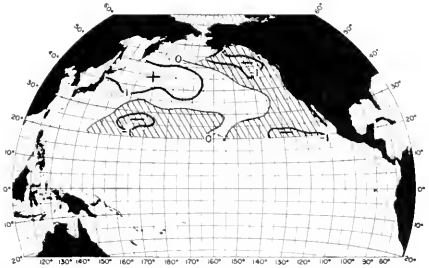

FIGURE 12.—Sea-surface temperature anomaly for November 1956. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (−) cells.

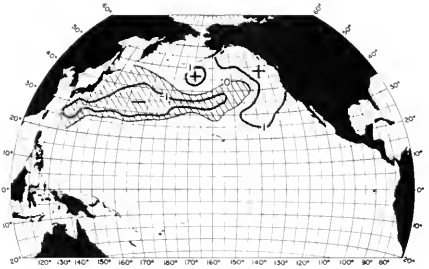

FIGURE 14.—Sea-surface temperature anomaly for June 1957. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (−) cells.

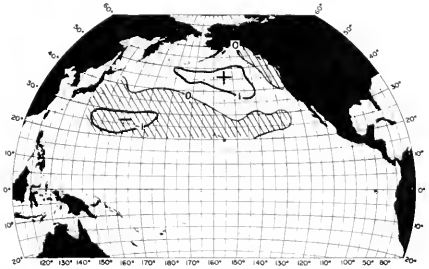

FIGURE 13.—Sea-surface temperature anomaly for March 1957. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (−) cells.

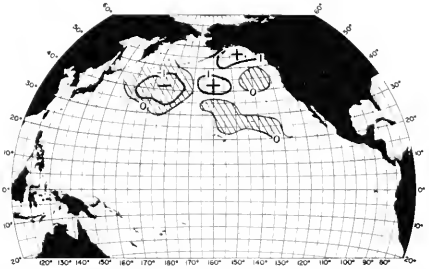

FIGURE 15.—Sea-surface temperature anomaly for August 1957. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (−) cells.

a single prominent cell which, in August 1957, was centered at about lat 40° N, long 175° E (Figure 15). This feature remained fairly steady through November 1957 (Figure 16), but all remnants of the cold anomalies in the east sector vanished.

The cell structure in the negative anomaly deteriorated at the beginning of 1958, but reformed in March with the cold cell east of its earlier position, at lat 30° N to 40° N, long 145° W to 170° W (Figure 17). Further fluctuations took place in the negative region until August 1958, when a dominant cold cell appeared at lat 35° N to 50° N, long 150° W to 175° W (Figure 18). The zone of positive anomalies along the North American coast was strongly developed during most of 1958 and 1959. However, the emphasis in the distribution shifted to the south after mid-1958, and the warm tongue reaching southwestward, south of lat 30° N, was most intense in the early months of 1959 (Figures 19 and 20).

Elsewhere the pattern tended to be somewhat weak and disorganized, and remained so until November 1959, when prominent cells, in which departures from normal exceeded 2° C, were evident in the central oceanic region of negative anomalies and in the positive coastal zone

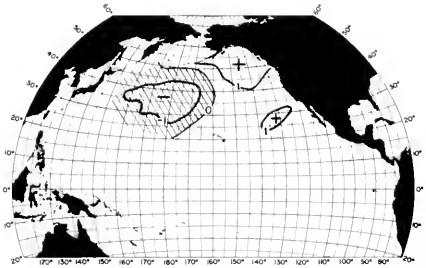

FIGURE 16.—Sea-surface temperature anomaly for November 1957. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (−) cells.

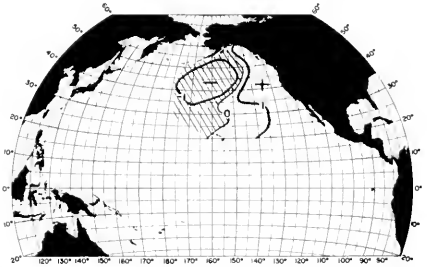

FIGURE 18.—Sea-surface temperature anomaly for August 1958. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or (−) cells.

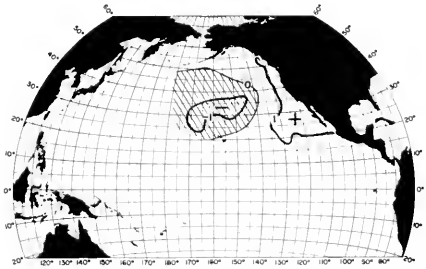

FIGURE 17.—Sea-surface temperature anomaly for March 1958. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (−) cells.

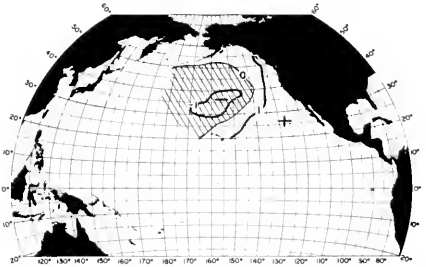

FIGURE 19.—Sea-surface temperature anomaly for January 1959. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (−) cells.

(Figure 21). The intensity and size of the warm cells diminished by February 1960 (Figure 22) and anomalies along the North American coast were generally weak, although mostly positive, through mid-1961.

The cold cell present in the west central Pacific in November 1959 had, by February 1960, pushed eastward to a new location, at lat 32° N to 48° N, long 155° E to 155° W (Figure 22). Thereafter it remained essentially within this region until the spring of 1961. However, the size and shape of the cold cell fluctuated considerably during this extended interval, as indicated in the charts for June 1960, November 1960, and February 1961 (Figures 23-25). This cold cell began to shift eastward in May 1961 and, by June, had reached a position at lat 32° N to 45° N, long 137° W to 160° W (Figure 26) where it remained through August. A new warm cell had appeared in the west sector at lat 30° N to 40° N, long 170° W to 165° E in June, but cannot easily be related to an impending transition in the anomaly field. It appeared to shift westward in July and August then vanished in September 1961, when the overall pattern collapsed.

The third and final phase in the progression of events began rather abruptly in October 1961

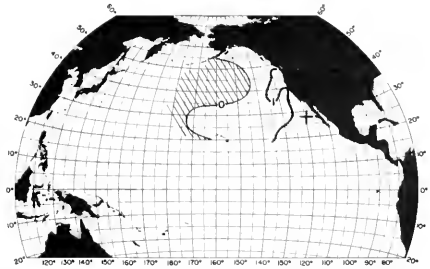

FIGURE 20.—Sea-surface temperature anomaly for March 1959. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (−) cells.

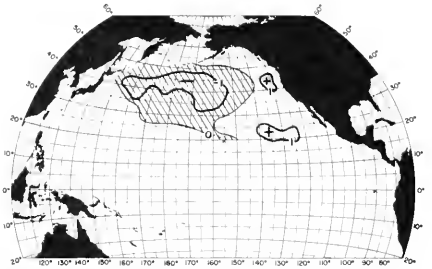

FIGURE 22.—Sea-surface temperature anomaly for February 1960. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (−) cells.

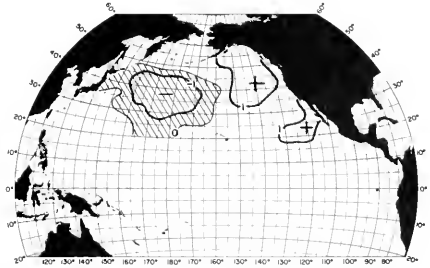

FIGURE 21.—Sea-surface temperature anomaly for November 1959. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (−) cells.

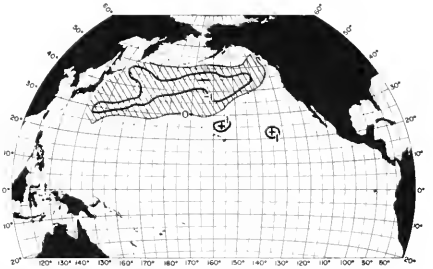

FIGURE 23.—Sea-surface temperature anomaly for June 1960. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (−) cells.

with a second reversal of the anomaly field in the central and eastern North Pacific Ocean (Figure 27). The principal feature of the new pattern was a warm cell which developed at lat 35° N to 45° N, long 145° W to 175° W. Departures from normal temperatures along the coastal zone and in the region to the southeast of the warm cell were weak but predominantly negative. In a broad sense, this distribution remained essentially undisturbed for nearly a year. The warm cell shifted eastward in January 1962 (Figure 28), diminished in size and intensity in March and April, but revived in June 1962 (Figure 29). A second warm cell was present at that time, but did not retain a separate identity for long. The original warm cell began a westward movement which, by September (Figure 30), returned it to the same location it occupied 11 months earlier in October 1961 (Figure 27).

MAINTENANCE OF PERSISTENT ANOMALIES

The examples described in the preceding section depict a recurring pattern in the distribution of sea-surface temperature anomalies. Schematically, the principal features consist of

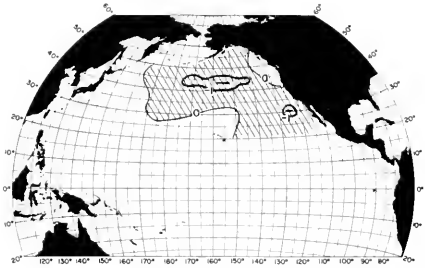

FIGURE 24.—Sea-surface temperature anomaly for November 1960. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (−) cells.

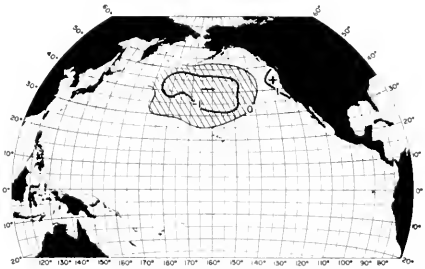

FIGURE 25.—Sea-surface temperature anomaly for February 1961. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (−) cells.

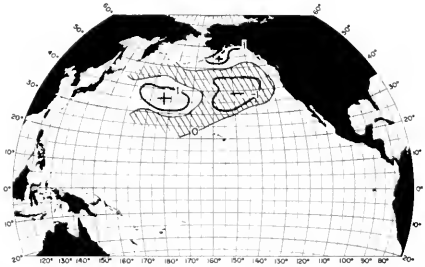

FIGURE 26.—Sea-surface temperature anomaly for June 1961. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (−) cells.

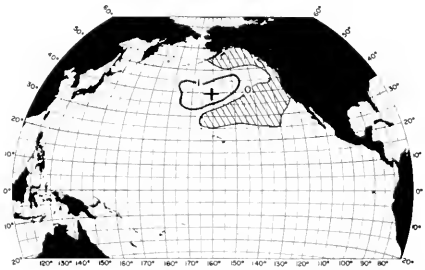

FIGURE 27.—Sea-surface temperature anomaly for October 1961. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (−) cells.

a dominant, quasi-stationary cell in a latitudinal belt of either positive or negative anomalies, located approximately between lat 30° N and 50° N, partially encircled by anomalies of opposite sign to the north, east, and south, in a zone contiguous with the North American coast. The dominant cell in this model is embedded in the North Pacific West Wind Drift Current. It reflects a wavelike displacement of the isotherms, which normally are very nearly zonal from about long 150° E to 140° W in the vicinity of lat 40° N.

The fact that this cell, which defines an area of maximum departure from normal in the temperature field, is not propagated eastward with the ocean current suggests the existence of a standing wave, or perturbation, in the current structure. Assuming this to be so, water entering the wave would turn north (or south) of its normal course, cutting across the normal isotherms and thereby causing anomalous local advection of high (or low) temperature. Downstream from the point of maximum excursion the water cuts back toward its original course and temperature conditions revert toward normal. Because the temperature gradient across the West Wind Drift Current is moderately strong, a small displacement of the isotherms

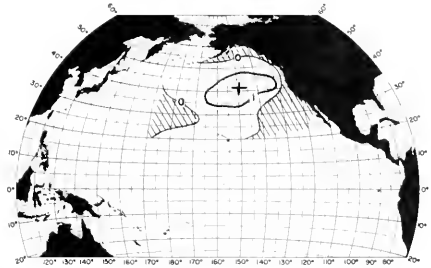

FIGURE 28.—Sea-surface temperature anomaly for January 1962. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (−) cells.

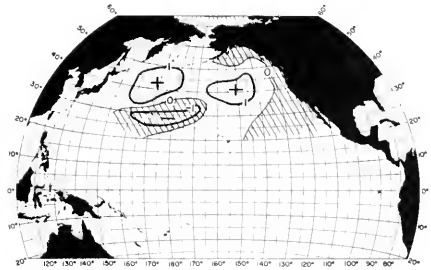

FIGURE 29.—Sea-surface temperature anomaly for June 1962. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (−) cells.

relative to the width of the current can create an anomaly of 1° C to 2° C. The amplitude of the perturbation in the current must, of course, be greater than that in the temperature field, since the water must adjust toward new equilibrium temperature appropriate to the local heat exchange processes as it changes latitude. Thus, a parcel of water following a northward deflection of the streamlines would arrive at the northern bend, or crest, of a standing wave with a temperature lower than it would have had if no deviation from zonal flow had occurred. Owing to the advective effect, it would nonetheless be warmer than an equivalent parcel of water

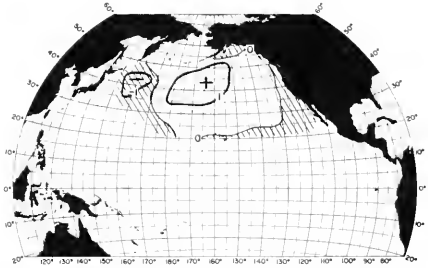

FIGURE 30.—Sea-surface temperature anomaly for September 1962. Hatched areas colder than normal. Heavy lines represent the 1° C anomaly contours which define warm (+) or cold (−) cells.

reaching the same location by a normal, zonal trajectory.

The existence of a perturbation upstream from the location where the West Wind Drift splits may have a significant effect on the proportional flow into the Alaska Gyre and the California Current. For example, in a wave formed by a northward deflection of the zonal current, water moving downstream from the crest would have a southward component which might favor more transport into the California Current. A strengthening of this current would cause the isotherms to adjust to the effects of increased cold advection and local heat exchange processes southward of their normal positions, creating a negative anomaly. Correspondingly, the reduced northward transport would decrease warm advection into the Gulf of Alaska and the isotherms would adjust to equilibrium positions farther south than normal, also creating a cold anomaly.

If the northward deflection in the foregoing example is replaced by a southward deflection, then, after passing the southern bend or trough of the wave, the water would approach the coast with a northward component, favoring more transport into the Gulf of Alaska. The balance between advection and local heat exchange would be established with the isotherms north of their normal positions, creating a warm anomaly.

In a comprehensive treatment of oceanographic survey data taken in 1955-58, Tully, Dodimead, and Tabata (1960) found the warm coastal anomaly of 1957-58 to be associated with increasing transport into the Alaska Gyre. They describe this condition in terms of a southward shift of the point of separation of flow from the West Wind Drift. From their study of the dynamic topography, they inferred that prior to the shift, in 1955 and 1956, most of the water approaching the coast south of lat 45° N entered the California Current. The fact that negative anomalies prevailed along the coastal zone in the latter half of 1955 and, to a lesser extent, in 1956 suggests that the point of separation in this period was farther north than usual.

Whether the partition of the West Wind Drift is influenced by upstream perturbations in the zonal flow structure cannot be established with certainty from the available survey data. The geopotential topography as presented by Dodimead, Favorite, and Hirano (1963) for the years 1955-59 does not reveal conclusive evidence of upstream wave structure. The physical evidence is limited to the anomalous characteristics of the surface temperature field which have already been discussed.

Some added perspective might be gained by looking briefly at the dynamic constraints applicable to a standing wave in the West Wind Drift. The average eastward current speed, U, for a wave of length L and lateral (north-south) extent D is given by Panofsky (1956) as follows:

$$U - C = \frac{2\Omega\cos\phi L^2}{4\pi^2 E}\left(\frac{1}{1 + L^2/D^2}\right)$$

where C is the wave speed (zero for a standing wave)

ϕ is the mean latitude

Ω is the angular velocity of the earth (7.292×10^{-5} sec^{-1})

E is the radius of the earth (6.37×10^{6} m).

This expression reduces to the Rossby wave equation for a uniform zonal stream on a rotating planet when the value of D approaches infinity.

In order to evaluate the right side of the above equation, we will assume dimensional similarity between the inferred wave and areas enclosed by the plus or minus 1° C anomaly contours for those cases where we presume a causal relation with the current structure. Estimates of the wave length L and of the ratio L/D were determined from rough measurements of the longitudinal and lateral extent of the warm cell present from October 1961 to September 1962. Excluding two extreme cases (April and July 1962) the longitudinal dimensions of the warm cell ranged between about 800 (March 1962) and 1600 (January 1962) nautical miles. The corresponding ratios of longitudinal to lateral extent for these particular cases were 1.3 and 2.0 respectively. Substitution of these values for L and L/D in the wave equation yields 35 and 85 cm/sec (approximately) for average current speed through a stationary wave. Of course, the areas enclosed by the 1° C anomaly contours presumably define only a portion of the hypothetical wave, and to substitute the dimensions of these areas for L and D would understate the theoretical current speed.

Current speeds in the West Wind Drift, computed from dynamic height anomalies, are generally less than 10 cm/sec (Dodimead et al, 1963). Data from drift bottles (Dodimead and Hollister, 1962) indicate current speeds up to 20 cm/sec in the same region. Thus, the theoretically computed results are too high, but considering the approximations used they are not altogether unreasonable.

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INDUCED SPAWNING OF THE NORTHERN ANCHOVY, *Engraulis mordax* GIRARD

RODERICK LEONG[1]

ABSTRACT

Anchovies were induced to mature their gonads by an artificial photoperiod of 4 hr light and 20 hr darkness at 15° C. Single injections of suspensions of salmon pituitary, carp pituitary, or a solution of human chorionic gonadotropin (HCG) promoted increase in egg diameters but did not induce spawning. Two injections, a first of HCG and the second 2 days later of either salmon pituitary or carp pituitary, induced spawning. At each spawning 6,000 to 16,000 eggs were collected, and 25% to 80% of the eggs hatched. Larvae grown from these eggs were morphologically similar to those caught in the sea.

An investigation was started at the National Marine Fisheries Service Fishery-Oceanography Center, La Jolla, Calif., in 1969, to examine methods for spawning the northern anchovy, *Engraulis mordax* Girard, under controlled laboratory conditions in order to supply eggs and larvae for experimental studies. The strategy for spawning anchovies in captivity was to provide an environment in which the fish would mature their gonads and subsequently to induce spawning through hormone treatment. The role of the environment and the use of hormone injections for inducing spawning in other species of fish has been well documented by Pickford and Atz (1957). This report describes a method for bringing the anchovy to ripeness and the effectiveness of various hormone treatments in inducing spawning. As far as is known this was the first successful attempt to artificially mature and spawn this pelagic fish in the laboratory.

MATERIALS AND METHODS

Anchovies, averaging 90 mm in length, were purchased from a San Diego bait dealer in March of 1969, transported to the laboratory, and held in circular plastic lined wading pools 4.6 m in diameter with 0.9 m of water. By the beginning of the injection trials in August of 1970, the fish had grown to an average length of 125 mm, at which length half of the fish should have been mature (Clark and Phillips, 1952). The fish were subjected to a photoperiod of 4 hr light (32 ft-c at the brightest spot on the surface of the water) and 20 hr dark (1 ft-c) for 3 months prior to the trials. Observations in the preceding year revealed that anchovies tend to mature more readily under relatively prolonged dark conditions. The tanks were constantly supplied with fresh seawater and the temperature was maintained at 15° C (Lasker and Vlymen, 1969). Much of the spawning of anchovies in nature occurs at or near this temperature (Ahlstrom, 1956). The fish were fed twice a day. In the first feeding, given at the beginning of the 4-hr light period, the fish were fed 6% of their live body weight in rotating daily rations of ground squid, ground anchovies, and brine shrimp. In the second feeding, given near the end of the light period, the fish were fed 1% of their body weight in trout chow.[2] Under this light, temperature, and food regimen approximately one-fourth of the fish developed gonads which weighed more than 6% of their body weight.

Three series of injection trials were conducted. In the first series the following dosages and types of injections were tested: 2.5, 5.0, and pituitary[3] prepared essentially by the method of 10.0 mg of salmon (*Oncorhynchus tshawytscha*)

[1] National Marine Fisheries Service Fishery-Oceanography Center, La Jolla, Calif. 92037.

[2] Ralston Purina trout chow, size 2. Reference to commercial products does not imply endorsement.

[3] Obtained through the courtesy of Dr. Irwin Haydock and the California Department of Fish and Game, Nimbus Fish Hatchery, Rancho Cordova, Calif.

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Haydock (1971), 2.5, 5.0, and 10.0 mg of commercial carp pituitary,[4] 1.0, 2.5, and 5.0 mg of deoxycorticosterone acetate (DOCA),[5] 1.0 mg of luteinizing hormone (PLH),[6] and 25, 50, and 100 international units (IU) of human chorionic gonadotropin (HCG).[5] The treatments were given in single 0.1-ml injections using a carrier of Holtfreter's solution (Emmel and Cowdry, 1964) in all cases except for DOCA where sesame oil was used. The suspensions of pituitary were prepared by triturating a weighed quantity in a small tissue grinder with enough liquid to form the proper concentration. The suspension was then pipetted into a small serum bottle where it could be withdrawn with an injection syringe. The injections were administered intraperitoneally with a 24-gauge needle between the pelvic fin and vent. Prior to injection the fish were anesthetized in 50 liters of water with 7 ppm quinaldine (Vrooman and Paloma, 1966).

Under each treatment 12 to 15 fish were injected. The sex and level of ripeness of living anchovies are difficult to distinguish and a portion of the fish in these trials were immature. Therefore it was necessary to inject this relatively large number of fish to increase the probability that some would be sufficiently developed to respond to hormone injections. Injected fish were placed in small holding tanks 1.2×1.2 m with 0.9 m of water. These tanks had running seawater and the temperature was also maintained at 15° C. Nets with 202-μ mesh were placed at the outflows of these tanks to collect eggs in the event of spawning. If no spawning occurred after 48 hr, the fish were stripped and fertilization attempted by the dry method (Davis, 1961). In this method the eggs and sperm are expressed, mixed, and left to stand in a dry container for 5 to 10 min before being placed in water. It was noticed in all trials that at least some males produced motile sperm.

The fish were killed after stripping and ovaries were removed from the females. Each ovary was teased apart and the major diameters of the most advanced eggs measured. The maximum diameters of injected fish were compared with the diameters of more than 500 captive uninjected females sampled during the previous 16 months to determine which of the treatments were effective in producing growth.

The effect of two injections of different hormones were examined in the second series of trials. The first injection was 50 IU of HCG and the second, given 48 hr later, was one of the following: 2.5 mg of salmon pituitary, 10.0 mg of carp pituitary, 5.0 mg of DOCA, 1.0 mg of PLH and 250 IU of gonadotropin from pregnant mare serum (PMS).[6] The methods of anesthetizing, injecting, and holding of fish were the same. If spawning did not occur within 48 hr after the second injection, the fish were stripped and fertilization attempted. In this and the final series of trials the fish were not killed after stripping and measurements of egg diameters were not made. Here the only criterion for success was spawning or production of viable eggs through stripping.

The effect of three injections of the same hormone was tested in the third and final series of trials. One group of fish was given three injections of 2.5 mg of salmon pituitary and another group three injections of 50 IU of HCG. The injections were spaced a day apart and the procedures were the same as described earlier. If no spawning occurred within 24 hr after the third injection, the fish were stripped and fertilization attempted.

RESULTS

None of the fish that were given a single injection spawned or produced viable eggs through stripping. Most of the stripped eggs were clumped, opaque, and apparently had not ovulated. Some of the treatment, however, produced noticeable increases in egg diameters. Table 1 shows the number of females under the various single-injection treatments and the number with and without eggs larger than 1.0 mm. The number of females was small in some

[4] Purchased from Stoller Fisheries, Spirit Lake, Iowa.
[5] DOCA and HCG purchased from Sigma Chemical Co., St. Louis, Mo.
[6] PLH and PMS purchased from Calbiochem, Los Angeles, Calif.

TABLE 1.—Female anchovies after various hormone injections having eggs larger than 1.0 mm in diameter. The occurrence of females with eggs larger than 1.0 mm is considered to be an indication of induced egg maturation. Of more than 500 captive uninjected fish sampled during the previous 16 months none had eggs larger than 1.0 mm. The average injected fish weighed about 25.0 g and measured 125 mm in length.

Type of injection	Dose/fish	No. females injected	No. females with eggs >1.0 mm
Salmon pituitory (*Oncorhynchus tshawytscha*)	2.5 mg	8	2
	5.0 mg	7	4
	10.0 mg	8	1
Commercial carp pituitary	2.5 mg	5	0
	5.0 mg	3	0
	10.0 mg	4	1
Human chorionic gonadotropin (HCG)	25 IU	4	1
	50 IU	8	1
	100 IU	6	0
Deoxycorticosterone acetate (DOCA)	1.0 mg	3	0
	2.5 mg	6	0
	5.0 mg	8	0
Luteinizing hormone (PLH)	1.0 mg	4	0

cases because most of the injected fish were males or had died through handling. Of the more than 500 females examined during the previous 16 months, none had eggs larger than 1.0 mm. The occurrence of females, Table 1, with eggs larger than 1.0 mm indicates that salmon pituitary, carp pituitary, and HCG are capable of promoting overnight growth of eggs. Salmon pituitary appeared to be the most potent for producing growth. The largest eggs observed were over 1.3 mm in diameter and within the size range, 1.23 to 1.5 mm, of naturally spawned eggs (Bolin, 1936). DOCA and PLH at the dosages tested were not effective in stimulating egg growth. The preponderance of fish with smaller eggs may be due to a low state of ovarian development at the time of injection.

In the second series of trials, HCG followed by DOCA, PLH, or PMS did not induce spawning and subsequent stripping produced only unovulated eggs which were not successfully fertilized. The combinations of HCG followed by salmon pituitary and HCG followed by carp pituitary induced spawning within 18 hr after the second injection. The fish spawned and fertilized the eggs themselves and large numbers of eggs were caught in the nets at the outflows of the tanks. Spawning was repeated several times with each of these two combinations of injections. The spawnings produced from 6,000 to 16,000 eggs with the percentage hatching varying from 25 to 80%. The larvae from these induced spawnings appeared morphologically normal and many were reared past 25 days by the methods of Lasker et al. (1970). The differences in the hatching percentage may be attributed to variation in the state of gonad development of parent fish at the time of injections.

The number of eggs collected suggests that only one or two females from any of the spawning groups contributed eggs. According to estimates by MacGregor (1968) female anchovies spawn almost 600 eggs per gram of fish. The average female in these trials weighed approximately 25 g and should have produced nearly 15,000 eggs. Only one or two females from any of the twice-injected groups extruded ovulated eggs upon stripping. Although the eggs were translucent and measured about 1.5 mm in diameter less than 10% hatched after being mixed with motile sperm.

In the final series of trials, three injections of salmon pituitary or three injections of HCG over a 3-day period failed to induce spawning. The stripped eggs were opaque and fertilization was not successful. These limited results suggest that the combination of HCG followed by salmon pituitary is more effective for induction of spawning than when these hormones are administered alone.

The results of these injection trials demonstrate that the northern anchovy can be induced to spawn in captivity and two effective treatments are HCG followed by salmon pituitary or HCG followed by carp pituitary after gonads are matured by a specific light-dark treatment of the fish. The induction of spawning of anchovies in the laboratory provides a practical way for supplying viable eggs for studies on larvae. In this study the time of spawning was controlled and eggs can probably be produced the year around if a large stock of fish is maintained. This was emphasized by the fact that the fish in this study were induced to

spawn during the late summer and fall months when anchovy eggs are virtually absent from the sea off San Diego. As far as is known, these are the first reported spawnings of *Engraulis mordax* in captivity and the first hormone-induced spawnings of engraulids.

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GILL RAKER APPARATUS AND FOOD SELECTIVITY AMONG MACKERELS, TUNAS, AND DOLPHINS

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ABSTRACT

Gill raker morphology and fork length were measured from 411 fish, representing eight species of scombrids and two species of coryphaenids (dolphin). For each species linear regressions passing through the origin were determined relating mean gill raker gap in millimeters (first gill arch) with fork length in centimeters (l), and log filtering area (first gill arch) with log fork length. Mean gill raker gaps equaled: *Auxis rochei*—0.0144l, *Katsuwonus pelamis*—0.0211l, *Auxis thazard*—0.0213l, *Thunnus albacares*—0.0344l, *Thunnus alalunga*—0.0365l, *Euthynnus affinis*—0.0386l, *Thunnus obesus*—0.0391l, *Sarda chiliensis*—0.0509l, *Coryphaena hippurus*—0.0650l, *Coryphaena equisetis*—0.0655l, and *Acanthocybium solanderi*—no gill rakers. Among the species gill raker gap was directly proportional to the number of gill rakers, but no relation occurred between mean gap and filtering areas. Gill raker gap differed markedly among species and lengths of fish. A 50-cm *K. pelamis*, a 30-cm *T. albacares*, and a 10-cm *Sarda orientalis* all had an estimated mean gap of 1 mm. Conversely the gaps of a 50-cm fish of each species were estimated to be ca. 1.0, 1.7, and 4.5 mm respectively.

Mean gill raker gaps from this study were compared with the percentage of crustaceans in stomachs of Central Pacific fishes based on literature records. Body sizes of fishes and squids in the stomachs were larger than crustaceans. Percent volumes that crustaceans contributed to the stomach content were inversely related to mean gaps (Kendall rank correlation coefficient, $\tau = -0.59$, $n = 16$, $P<0.001$). Partial correlation indicated that gap was more important than fork length in predicting the quantity of crustaceans. Thus, the gill raker gap was related functionally with the quantity of smaller organisms in the stomachs. Presence of euphausids in stomachs of *K. pelamis* and their absence in *T. albacares* from the eastern tropical Pacific may result from the small size of euphausids and the smaller gill raker gaps of *K. pelamis* relative to *T. albacares*. Gill raker gap and the maximum distensibility of the esophagus would set physical limits on the size of food eaten. The diverse fauna assemblage of crustaceans, fishes, and squids within this size range has masked to a great extent the selective feeding that does occur among scombrids and coryphaenids on the basis of food size.

Most scombrid fishes have a varied diet that includes numerous crustaceans, cephalopod molluscs, and fishes. The Indian mackerel, *Rastrelliger kanagurta* (Cuvier), even eat phytoplankton (Bhimachar and George, 1952). The high diversity of organisms in their stomach contents has generated the opinion that scombrids are nonselective feeders, preying upon anything they encounter. Coryphaenid fishes, dolphins, eat fish predominantly.

Yet selectivity does exist in food habits of scombrids. Within a species, larger fish contain relatively fewer crustaceans and more fishes. Crustaceans constituted 44% of the stomach volume of skipjack tuna, *Katsuwonus pelamis* (Linnaeus), shorter than 50-cm fork length but only 1.5% of the volume for fish longer than 60 cm (Yuen, 1959). Similarly, crustaceans constituted 35% of the stomach volume of yellowfin tuna, *Thunnus albacares* (Bonnaterre), shorter than 130 cm but only 1% for those longer than 130 cm (Reintjes and King, 1953). Reintjes and King suggested that these differences might result, as the fish grew, from a change in food preference or a change in the ability to search out and capture larger, more mobile prey (fishes). Another consideration, in our view, is that larger predators have a reduced ability to catch small prey (crustaceans).

Prevention of food loss through the opercular gap is generally recognized as the primary function of gill rakers. Species with more closely

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spaced gill rakers are more likely to feed on plankton than those with more widely spaced rakers (Suyehiro, 1942; Yasuda, 1960a; Brooks and Dodson, 1965; Kliewer, 1970).

This paper (1) quantitatively describes the gill raker apparatus of certain scombrids and coryphaenids with respect to the gap between gill rakers and the filtering area of the first gill arch, (2) compares differences in gill raker gap among species and lengths of fish, and (3) considers the proposition that observed inter- and intraspecific variations in the diet are associated functionally with the morphometrics of the gill raker apparatus.

MORPHOMETRY OF GILL RAKER APPARATUS

Gill raker morphometry and fork length were measured from 411 fish, representing eight species of scombrids and two species of coryphaenids. Albacore, *Thunnus alalunga* (Bonnaterre), were from the commercial longline fishery operated from American Samoa, the Pacific bonito, *Sarda chiliensis* (Cuvier), were from waters off Palos Verdes, Calif., and chub mackerel, *Scomber japonicus* Houttuyn, were from the Honolulu fish market. All other specimens were from Hawaiian waters and were caught with pole and line or longline by commercial fishermen or on numerous cruises of the research vessel *Charles H. Gilbert* of the Bureau of Commercial Fisheries Biological Laboratory, Honolulu (now National Marine Fisheries Service Hawaii Area Fishery Research Center).

Measurements were from the first right gill arch of fresh or thawed specimens. The arch was removed from the fish and extended by pulling the upper and lower branches apart until the rakers were stiffly erect. Gaps between adjacent rakers (Figure 1) were measured at the base of the rakers by expanding a vernier caliper until the two gill rakers began to spread apart. Arch length and gill raker length were also measured with the caliper (Figure 1). Depending on the species, six to nine gaps and six to eight gill raker lengths spaced along the arch

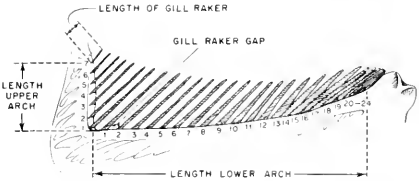

FIGURE 1.—Diagram of the first right gill arch of a scombrid as viewed from oral chamber showing the morphometric measurements. Numbers indicate particular rakers.

were obtained from scombrids and three gaps and five gill raker lengths from coryphaenids. Mean gap was the average of those measured along the arch. A gap near the middle of the lower arch was also used to represent gap width in the primary filtering area. Filtering area was calculated from average length of gill rakers and length of the arch. Lower and upper arch filtering areas were computed separately and summed.

DESCRIPTION

Gill rakers of the first arch of most scombrids were conspicuous and well developed. Inner edges of the rakers of most species were covered with numerous short, spiny protuberances. For *S. japonicus*, these spines were thin, about as long as the gill raker gap, and evenly spaced to form a finer sieve between adjacent gill rakers. The other three arches of scombrids lacked gill rakers, but smaller rakerlike processes on the inner faces of the all arches projected posteriorly to the adjacent arch forming a sieve. Inner edges of these processes had short, spiny protuberances similar to the gill rakers.

Rakers were articulated so that they became stiffly erect forming a parallel row of blade-shaped rakers when the acute angle between the upper and lower arch was expanded toward 90 degrees. In the branchial chamber the tips of the rakers extended to the inner surface of the flared gill cover.

The wahoo, *Acanthocybium solanderi* (Cuvier), has no gill rakers, but most scombrids have more than 20 elongated rakers—*K. pelamis* in

our samples had 53 to 64. Longest rakers were near the joint between the upper and lower branches of the arch. They became progressively shorter toward the ends of the arch. For example, a *K. pelamis* 50 cm long had gill rakers 21 mm long at the joint but only 2 and 8 mm at the ends of the upper and lower branches, respectively. The largest gap (1.8 mm) was near the center of the lower branch. Gaps were smaller on the upper branch than lower branch and were most narrow at the ends of the arch (0.2 mm and 0.9 mm for the upper and lower branches). Often the gap between the first raker of the upper and the first raker of lower arch was as great as the widest gap on the lower arch.

Most of the filtering area of scombrids was confined to the lower branch of the gill arch. The lower branch comprised 73 to 80% of the total. The filtering area of coryphaenids was essentially restricted to the lower arch. Dolphin, *Coryphaena hippurus* Linnaeus, had no rakers on the upper arch, pompano dolphin, *Coryphaena equisetis* Linnaeus, had only one.

Gill rakers of the two coryphaenids were shorter and more uniform in length than those of scombrids. The longest gill raker from a 55-cm *C. equisetis* was only 9 mm contrasted

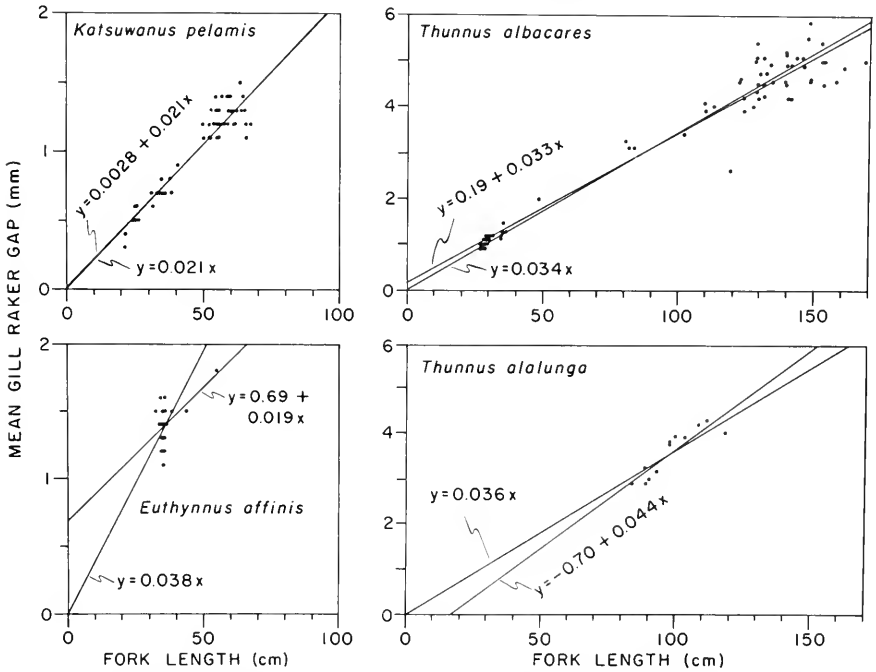

FIGURE 2.—Relation between mean gill raker gap and fork length showing the advantage of using regression through the origin for predicting mean gill raker gap especially when sample sizes are small and restricted in length range.

to 21 mm from a 50-cm *K. pelamis*. Even the longest raker of a 125-cm *C. hippurus* was only 16 mm—shorter than that of a 50-cm *K. pelamis*.

SIZE AND SPECIES COMPARISONS

METHODS

Linear regressions relating gill raker gap to fork length and log filtering area to log fork length were computed for each species. Regressions were computed once about the mean, and a second time, were forced to pass through the origin. The latter procedure was used because the ranges of fork lengths of some species were not sufficient to obtain reasonable equations (Figure 2).

Both *K. pelamis* and *T. albacares* were represented by large samples that included small and large specimens. Their regressions of gill raker gap on fork length passed close to the origin even when not forced to do so; the y-intercept was 0.00 mm for *K. pelamis* and 0.19 mm for *T. albacares* (Figure 2). In contrast, kawakawa, *Euthynnus affinis* (Cantor), and *T. alalunga* were represented by small samples that did not include small specimens. Regressions extrapolate outside the size ranges represented in our samples, the regressions forced to pass through the origin were used for all computations of gill raker gap.

The same reasoning was used for the relations between log filtering area and log fork length. In this case, the zero-zero intercept was equivalent to 1 cm fork length and 1 mm^2 filtering area rather than zero fork length and zero filtering area. Since most comparisons made later were for fish at least 35 cm long with filtering areas near 100 mm^2, errors owing to the position of the intercept were believed negligible.

SIZE AND SPECIES COMPARISONS

Linear regressions passing through the origin that relate gill raker gap to fork length and log filtering area to log fork length are presented in Table 1 along with the numbers and lengths of fishes measured.

Mean gill raker gap increased with fork length and was equal to 1.4 and 6.6% of fork length for frigate mackerel, *Auxis rochei* (Risso), and *C. hippurus*, respectively. Gill raker gap in the middle of the lower branch was usually 1.0 to 1.2 times the mean gill raker gap except for

TABLE 1.—Linear regressions passing through the origin that relate mean gill raker gap to fork length and log filtering area to log fork length and the number and length of fish measured.

Species	Number of fish measured	Regressions of gap (G) and fork length (l)	Fork length		G (mm)	Standard error of estimate for G (mm)	Regressions of log filtering area (log A) and log fork length (log l)	Mean		Standard error of estimate for Log A (mm^2)
			Mean (cm)	Range (cm)				Log l (cm)	Log A (mm^2)	
Sarda chiliensis	8	$G = 0.0509\ l$	50.13	38.7- 58.8	2.56	0.27	log A = 1.73 (log l)	1.7001	2.9246	0.0584
Auxis thazard	16	$G = 0.0213\ l$	31.40	25.1- 35.7	0.67	0.10	log A = 1.79 (log l)	1.4969	2.6730	0.0393
Auxis rochei	11	$G = 0.0144\ l$	30.24	29.2- 32.9	0.44	0.06	log A = 1.78 (log l)	1.4706	2.6291	0.0192
Euthynnus affinis	25	$G = 0.0386\ l$	36.29	33.4- 54.6	1.41	0.29	log A = 1.82 (log l)	1.5598	2.8421	0.0234
Katsuwonus pelamis	63	$G = 0.0211\ l$	47.60	21.0- 67.5	1.00	0.10	log A = 1.83 (log l)	1.6776	3.0135	0.0697
Thunnus alalunga	12	$G = 0.0365\ l$	98.56	84.0-118.8	3.59	0.25	log A = 1.81 (log l)	1.9937	3.6086	0.0343
Thunnus albacares	74	$G = 0.0344\ l$	94.86	27.1-166.9	3.31	0.36	log A = 1.78 (log l)	1.9771	3.3468	0.0405
Thunnus obesus	82	$G = 0.0391\ l$	132.96	75.2-175.3	5.26	0.60	log A = 1.85 (log l)	2.1236	3.9058	0.0449
Coryphaena equiselis	38	$G = 0.0655\ l$	94.90	30.8- 58.7	6.16	0.54	log A = 1.39 (log l)	1.9773	2.7339	0.0704
Coryphaena hippurus	68	$G = 0.0650\ l$	40.52	63.2-126.4	2.69	0.34	log A = 1.36 (log l)	1.6077	2.1716	0.0727

of gill raker gap on fork length for these two species did not closely approach the origin (Figure 2); we believe these equations would also have had y-intercepts near 0.0 mm if lengths of our specimens had been more evenly distributed. Since some comparisons were made that *A. rochei* (1.3) and *K. pelamis* (1.4). Mean gap increased in direct proportion to fish length; i.e., if length doubled, gap also doubled.

Filtering area increased as the 1.4 to 1.8 power of fork length. When these regressions were not forced to pass through the origin, the filter-

ing area increased as the 2.2 power of fork length for *K. pelamis* and the 1.9 power for *T. albacares*. Forcing the regressions to pass through the origin may have decreased the slope.

To facilitate comparison of different species, the mean gap and filtering area were computed from the regression in Table 1 for fish with a fork length of 35 cm. These are listed in Table 2 in order of decreasing number of gill rakers, increasing gap, and decreasing filtering area.

As expected, the number of rakers and gill raker gap were closely related (Table 2). Lack of complete correspondence may have resulted from differences in the thickness of gill rakers, differences in the length of the gill arch, or both.

Among scombrids no relation was evident between filtering area and number of rakers or between filtering area and mean gill raker gap (Table 2). Apparently, the length of raker was an important variable determining differences

TABLE 2.—Scombrid and coryphaenid species (35-cm fork length) listed in order of increasing numbers of gill rakers, and decreasing mean gill raker gap and filtering area.
(Data on *S. orientalis* from one fish, *S. japonicus* from two fish.)

Rank	Mean number of rakers (n)	Species	Mean gill raker gap (mm)	Species	Filtering area (mm^2)	Species
12	8	*Coryphaena hippurus*	3.3	*Sarda orientalis*	--	*Scomber japonicus*
11	10	*Coryphaena equisetis*	2.3+	*Coryphaena equisetis*	685	*Thunnus obesus*
10	11	*Sarda orientalis*	2.3—	*Coryphaena hippurus*	650	*Euthynnus affinis*
9	25	*Sarda chilensis*	1.8	*Sarda chilensis*	620	*Thunnus alalunga*
8	26	*Thunnus obesus*	1.4	*Thunnus obesus*	530	*Katsuwonus pelamis*
7	29	*Thunnus alalunga*	1.4	*Euthynnus affinis*	570	*Auxis thazard*
6	30	*Thunnus albacares*	--	*Scomber japonicus*	550	*Auxis rochei*
5	31	*Euthynnus affinis*	1.3	*Thunnus alalunga*	450	*Sarda chilensis*
4	37	*Scomber japonicus*	1.8	*Thunnus albacares*	410	*Thunnus albacares*
3	40	*Auxis thazard*	0.74	*Auxis thazard*	--	*Sarda orientalis*
2	45	*Auxis rochei*	0.74	*Katsuwonus pelamis*	135	*Coryphaena hippurus*
1	58	*Katsuwonus pelamis*	0.51	*Auxis rochei*	120	*Coryphaena equisetis*

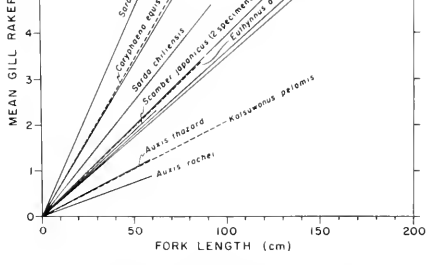

FIGURE 3.—Comparison of the mean gill raker gap and fork length relationship for various scombrid and coryphaenid fishes. Lengths shown approximate ranges known for each species.

in filtering area among species. Coryphaenids had a larger gill raker gap and smaller filtering area than any scombrid except striped bonito, *Sarda orientalis* (Temminck and Schlegel).

Among scombrids 35 cm long, *Sarda* had the largest gaps (1.8-3.3 mm) and *Auxis* and *Katsuwonus* the smallest (0.51-0.74 mm). *Thunnus*, *Euthynnus*, and *Scomber* had intermediate gap widths (1.2-1.4 mm). Among *Sarda*, *Auxis*, and *Thunnus* represented in our samples, species within genera had more similar gill raker gaps than those in different genera. On this basis alone food habits for fish of the same length would be expected to be more similar within genera than among genera.

Mean gill raker gap differed markedly with species and length of fish (Figure 3). For example, a 50-cm *K. pelamis*, a 30-cm *T. albacares*, and a 10-cm *S. orientalis* all had a mean gill raker gap of approximately 1 mm. Conversely, gill raker gaps of these three species differed markedly at the same fork length. Gaps of 50-cm *K. pelamis*, *T. albacares*, and *S. orientalis* were ca.

TABLE 3.—The average size of individual crustaceans, squids, and fishes in the stomachs of scombrids from the central Pacific.

Species	Volume of individual organisms (ml)			Volume of food analyzed (ml)	Source
	Crustaceans	Squids	Fishes		
Thunnus albacares	0.3	4.8	6.4	44,680	King & Ikehara (1956)
Thunnus obesus	0.6	9.5	8.2	22,297	King & Ikehara (1956)
Thunnus albacares	0.2	3.8	4.6	52,336	Reintjes & King (1953)
Katsuwonus pelamis	0.2	4.3	3.7	13,974	Waldron & King (1963)
Mean (unweighted)	0.3	5.6	5.7		

1.0, 1.7, and 4.5 mm, respectively. Selectivity of the gill raker apparatus would vary with gill raker gap, a function of both species and length of the fish. Thus, a small *T. albacares* and a large *K. pelamis* should have more similar diets than a small and a large *T. albacares*. Any number of such predictions can be generated from Figure 3. A fish with smaller mean gap, regardless of its species or length, would be expected to be more planktivorous.

RELATION BETWEEN GILL RAKER GAP AND DIET

Stomach-content data from published literature from the central Pacific were compared with the mean gill raker gaps reported here to test the hypothesis that fish with a finer gill raker gap have a greater proportion of smaller organisms (crustaceans) in their diet.

Crustaceans in the diet of scombrids from the central Pacific were smaller than were the other major food organisms (squids and fishes) (Table 3). The volume of individual, partially digested crustaceans in the stomachs of five species averaged 0.3 ml whereas individual, partially digested squids and fishes averaged 5.6 and 5.7 ml, respectively. The much smaller body size of the crustaceans was not likely the result of differential digestion, especially since the exoskeleton of crustaceans, if anything, might be expected to slow, rather than accelerate, digestion (Pandian, 1967).

For comparison with gill raker data, the percent volumes of the stomach content comprised by crustaceans, squids, or fishes are presented in Table 4 for five scombrids and one coryphaenid. Only stomach data from the central Pacific were used because differences in typical body size of crustaceans in scombrid stomachs from other regions would have invalidated these

TABLE 4.—Food of scombrid and coryphaenid fishes from the central Pacific divided into the percentages of the stomach volume that were crustaceans, squids, or fishes. The median fork length of fishes in the sample is also given along with the literature source for the data.

Species	Stomach content (percent volume)			Fork length		Number of stomachs	Literature source
	Crustaceans	Squids	Fishes	Median (cm)	Range (cm)		
Acanthocybium solanderi	0	0	100	111	104-123	3	Tester & Nakamura (1957)
Acanthocybium solanderi	0	--	--	--	54-198	235	Iversen & Yashida (1957)
Euthynnus affinis	8	0	92	49	31-67	32	Tester & Nakamura (1957)
Katsuwonus pelamis	44	--	40	44	39-49	>25	Yuen (1959)
Katsuwonus pelamis	4.0	23	72	47	33-60	305	Waldron & King (1963)
Katsuwonus pelamis	67	7	26	50	40-61	67	Tester & Nakamura (1957)
Katsuwonus pelamis	25	--	70	55	50-60	>25	Yuen (1959)
Katsuwonus pelamis	3.7	19	74	73	60-89	254	Waldron & King (1963)
Katsuwonus pelamis	1.5	--	91	73	62-84	>25	Yuen (1959)
Thunnus albacares	45	14	33	80	53-100	544	Reintjes & King (1953)
Thunnus albacares	39	9	49	115	100-130	205	Reintjes & King (1953)
Thunnus albacares	1.7	29	65	135	85-140	188	King & Ikehara (1956)
Thunnus albacares	3	4	93	140	130-168	26	Reintjes & King (1953)
Thunnus albacares	0.8	30	60	148	140-175	251	King & Ikehara (1956)
Thunnus obesus	2.3	26	70	128	75-140	63	King & Ikehara (1956)
Thunnus obesus	1.4	36	58	158	140-200	103	King & Ikehara (1956)
Coryphaena hippurus	1.6	2	97	81	42-121	52	Tester & Nakamura (1957)

TABLE 5.—Percent crustaceans by volume in the stomachs, median fork length, mean gill raker gap, and species of fish. (Ranks are from smallest to largest and ordered by the percentage of crustaceans in the stomachs.)

Species	Crustacea		Median fork length		Mean gill raker gap	
	%	Rank	(cm)	Rank	(mm)	Rank
Acanthocybium solanderi	0	1	111	10	0	16
Thunnus albacares	0.8	2	148	15	5.2	13
Thunnus obesus	1.4	3	158	16	6.3	15
Katsuwonus pelamis	1.5	4	73	6	1.5	5
Coryphaena hippurus	1.6	5	81	9	5.3	14
Thunnus albacares	1.7	6	135	13	4.7	10
Thunnus obesus	2.3	7	128	12	5.1	12
Thunnus albacares	3	8	140	14	4.9	11
Katsuwonus pelamis	3.7	9	73	6	1.5	5
Katsuwonus pelamis	4.0	10	47	2	0.99	2
Euthynnus affinis	8	11	49	3	1.9	7
Katsuwonus pelamis	25	12	55	5	1.2	4
Thunnus albacares	39	13	115	11	4.0	9
Thunnus albacares	44	14	80	8	2.8	8
Katsuwonus pelamis	45	15	44	1	0.92	1
Katsuwonus pelamis	67	16	50+	4	1.0	3

analyses. The galatheids and portunids dominating the crustaceans found in *T. albacares* stomachs in the eastern tropical Pacific (Alverson, 1963) are much larger (Longhurst, 1967; Jerde, 1967b) than the typical crustaceans from the stomachs of central Pacific scombrids given in Table 3. Also, data were not used if fewer than 25 stomachs had been examined. None of the 238 *A. solanderi* contained crustaceans and 0% crustaceans in the stomach was considered a reasonable estimate for any larger *A. solanderi*. The median or midrange fork length of fish was determined for each set of stomach data. Then mean gill raker gaps for fish of those species and length were estimated with the regressions from Table 1. Data on median fork length, mean gill raker gap, and percent crustaceans by volume in the stomach are presented in numerical and ranked form in Table 5.

Percent volumes that crustaceans contributed to the stomach content were inversely related to mean gill raker gap (Figure 4a) (Kendall rank correlation coefficient, $\tau = -0.59$; $n = 16$; $P < 0.001$) and to fork length (Figure 4b) (Kendall rank correlation coefficient, $\tau = -0.45$; $n = 16$; $P < 0.01$). Several notable exceptions occurred in the relation with fork length (Table 5, Figure 4b). *C. hippurus*, 81 cm long, contained 2% crustaceans while *T. albacares*, 80 cm long, contained 45% crustaceans. *T.* albacares, 135 cm long, also contained 2% crustaceans. Not unexpectedly, *C. hippurus*, 81 cm long, and *T. albacares*, 135 cm long, both had mean gill raker gaps near 5 mm whereas the 81-cm *T. albacares* had a smaller mean gill raker gap near 3 mm. The somewhat closer correspondence of percentage of crustaceans to gill raker gap than to fork length can be observed by comparing Figures 4a and 4b or by comparing the associated probabilities of no correlation ($<.01$ versus $<.001$).

Kendall partial rank correlation coefficients were computed to determine the association between percent crustaceans in the stomach and gill raker gap, with the effect of fork length held constant. The partial correlation coefficient between percent crustaceans and gap, independent of variation in fork length, was —0.43 while the partial correlation between percent crustaceans and fork length independent of variations in gap, was only —0.05. Thus, although fork length was correlated with the percent crustaceans, this correlation resulted from the association between gill raker gap and fork length. Gill raker gap was the important variable correlated to percent crustaceans in the diet.

Data on percent crustaceans in the stomach by volume were also presented for *K. pelamis* and *T. albacares* of various size by Alverson (1963) and for *K. pelamis* and blackfin tuna, *Thunnus atlanticus* (Lesson), by Suarez Caabro

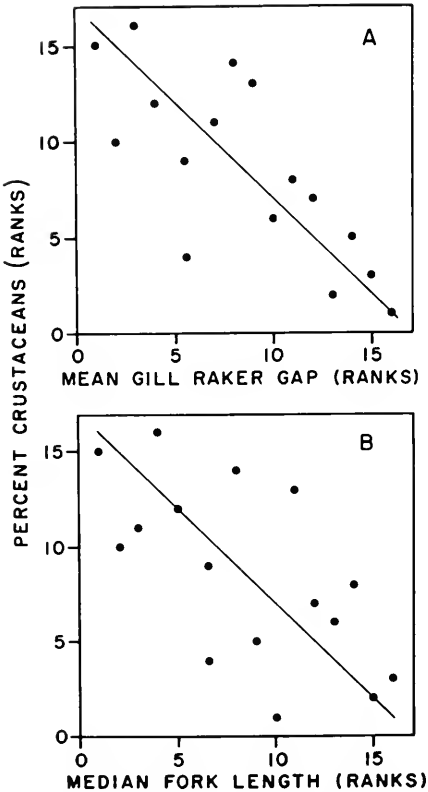

FIGURE 4.—Relation between percentage crustaceans by volume of diet (ranked) and the (a) mean gill raker gap of a fish (ranked) and (b) fork length of fish (ranked). The diagonal line depicts a perfect inverse relationship.

and Duarte Bello (1961). These were not used in the present analysis because sample sizes were fewer than 25 fish or because the size of the individual crustaceans was unavailable. Regardless, larger *K. pelamis* in both studies contained less crustaceans. However, larger *T. albacares* in Alverson's study tended to eat more crustaceans than did smaller specimens. This may have been because the crustaceans in their diet were relatively large.

Alverson's paper also presents a good example of selectivity among crustaceans that may be based on size of gill raker gaps. *K. pelamis* and *T. albacares* from the same areas had markedly different diets. Crustaceans contributing the greatest volume to the stomachs were galatheids and portunids for *T. albacares* but euphausids for *K. pelamis*. Euphausids were rare in stomachs of *T. albacares* even when common in the micronekton (Blackburn, 1968). Galatheids and portunids (Longhurst, 1967; Jerde, 1967b) are typically larger in size than euphausids (Jerde, 1967a). The small euphausids were not important in stomachs of *T. albacares* (i.e., 1% of the volume) in any of the areas of the eastern tropical Pacific studied by Alverson (1963), but the larger galatheids and portunids were important in the stomachs of *K. pelamis* from certain areas. The above observations would be the predictions from gill raker gaps—*T. albacares* have broader gaps than *K. pelamis* and would not be expected to capture the smaller crustaceans.

The major hypothesis under investigation in the present study was that the quantity of smaller organisms (crustaceans) eaten should be related to the selectivity of the gill raker apparatus. The above correlations on central Pacific data, although only crude in nature, lend support to this idea. A more definitive test would require extensive data on the size of food organisms and the diet of scombrids over more narrow length ranges than are available from the published literature.

Even though the structure of the gill raker apparatus ultimately determines the smallest size of prey, it is possible that actual selection of fishes is made prior to ingestion (Ivlev, 1961; Galbraith, 1967). Galbraith believed that the gill rakers of yellow perch, *Perca flavescens* (Mitchill), and rainbow trout, *Salmo gairdneri* Richardson, could have retained smaller zooplankton than were typically found in their stomachs. These species ate only larger *Daphnia* even though numerous smaller ones were in the zooplankton.

Several authors have pointed out that fish tend to select the largest food organisms available to them (Hayashi, 1956, as cited in Yasuda, 1960b; Ivlev, 1961; Brooks, 1968). The large mouth of larval scombrids facilitates capture of large copepods at first feeding and contributes to their rapid early growth rates (Shirota, 1970). The responsiveness of at least one scombrid to food is influenced by the size of the food organism—*K. pelamis* ate whole shrimp and squid at the beginning of a feeding, but as they became sated, they would only eat cut-up pieces of smaller size (Nakamura, 1962). Feeding behavior of Atlantic mackerel, *Scomber scombrus* Linnaeus, (Sette, 1950) and northern anchovy, *Engraulis mordax* Girard, (Leong and O'Connell, 1969) changes with the size of food. When small food is present, they open the mouth wide and flare the opercles in a filter feeding mode, but with larger food they make individual biting attacks. *S. japonicus* eats food smaller than would be predicted by gill raker gap (Hiyama and Yasuda, 1957). The spiny process we observed on the rakers of *S. japonicus* probably form an even finer sieve than is formed by the rakers themselves. Regardless of the mode of selection (anatomical, behavioral, or perceptual), the selective capabilities of scombrids and coryphaenids would appear to be correlated with the anatomy of the gill raker apparatus.

An individual scombrid is able to prey on organisms differing greatly in size. It is capable of engulfing and retaining crustaceans, small fishes, and squid by means of a well-developed gill raker apparatus. It is also capable of pursuing, capturing, and ingesting fast-moving fishes and squids, provided they are not too large to be swallowed whole. The gill raker gap and maximum distensibility of the mouth and esophagus then would be expected to set limits on the range of food sizes eaten by scombrids. Within this size range a diverse faunal assemblage exists in the sea that includes numerous species of crustaceans, fishes, and molluscs. The diversity of species in the size range consumed by an individual scombrid has, to a great extent, masked the selectivity that does occur. The present paper provides some evidence for selection of organisms above a minimum size determined by the magnitude of gill raker gaps.

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NATURE OF FREE RADICALS IN FREEZE-DRIED FISHERY PRODUCTS AND OTHER LIPID-PROTEIN SYSTEMS

WILLIAM T. ROUBAL[1]

ABSTRACT

The electron paramagnetic resonance spectrometer makes it possible to detect and study radicals which are produced during lipid peroxidation in freeze-dried lipid-protein systems. In such systems, two general types of resonances are observed. By studying effects of added antioxidants, added autoxidizable lipid, and type of substrate, it is possible to differentiate and characterize the two signals which are observed. It is postulated that the immobilized radicals observed in dry systems are the same as those that are responsible for damage at the molecular level in oxygenated lipid-protein-water emulsions, stored meats of normal moisture content, and in antioxidant deficiency states in man and animals.

Fish from the sea are rich in polyunsaturated fatty acids. Furthermore, when fish are processed as a foodstuff, the unsaturated fatty acids will, if unprotected, readily undergo lipid peroxidation.

In the presence of proteins, enzymes, nucleotides and other classes of biological materials, lipid peroxidation is radiomimetic, that is, it produces similar if not the same effects as ionizing radiation—a damage mechanism which is known to be mostly free radical in nature. Accordingly, such materials as freeze-dried fish tissue, dried fish meals, and protein concentrates incompletely freed of residual unsaturation, are all prone to undergo various deteriorative changes as a consequence of lipid peroxidation.

Although much emphasis has been placed in the past, and continues to be at present, on the participation of radicals in events leading to damage, radicals of the oxy or peroxy type have not been directly characterized by electron paramagnetic resonance (EPR) spectroscopy in living systems or in emulsions for in these the steady state concentration of radicals is universally low. Nevertheless, the EPR method remains as the one method best suited for characterizing radicals.

This paper discusses recent research employing systems which, for the first time, are favorable for the detection and study of EPR signals which arise with the onset of lipid oxidation. Mechanisms for the formation of radicals as well as reactions of radicals themselves are discussed.

MATERIALS AND METHODS

Freeze-dried and isopropyl-extracted rockfish myofibrillar protein, and freeze-dried rockfish sacroplasmic protein were provided by the NMFS Technological Laboratory in Seattle. In addition, a polyunsaturated fatty acid (PUFA) concentrate (75% C22:6 + 25% C22:5) was also provided by this laboratory. Freeze-dried human serum albumin (Grade III) and bovine serum albumin (BSA; crude powder) were obtained from Sigma. Freeze-dried silver salmon light flesh was prepared from a slurry of fresh fillet. All freeze-dried materials were stored at —60° C in the dark under nitrogen prior to exposure to air. Lipid-protein mixtures were prepared merely by thoroughly mixing the C22:6 concentrate with protein, usually in a ratio of 2:1 (protein to lipid) by weight. All oxidations were conducted in air at room temperature. All EPR studies were conducted at room temperature according to the procedures of Roubal (1970).

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RESULTS AND DISCUSSION

ELECTRON MIGRATION IN PROTEINS

As a base for comparing EPR signals in processed food materials, it will be instructive at this point to consider briefly the mechanism(s) which may be operative (disregarding for the moment, lipid oxidation) in the production of the observed signals in *carefully* freeze-dried tissue. In recent years, considerable attention has been given to the idea of the migration of energy over comparatively long distances in cells; such transfer of electrons is implicated in the process of mutation by ionizing radiation, in the process of nerve conduction, and in certain biochemical photo dissociations. There are problems of a theoretical nature associated with such hypotheses, and these are reviewed in the discussions of Blyumenfel'd (1957).

Terenin (1947) and Terenin and Krasnovskii (1949) have criticized Szent-Györgyi's hypothesis that electrons move along the protein polypeptide backbone in conductivity bands analoguous to the movement of electrons in conductivity bands of semiconducting metals. Although polypeptide chains exhibit properties reminiscent of conjugated unsaturation, Terenin poses the interesting observation that some 70 kcal mole, a large amount of energy, would be required to mobilize electrons; evidently at usual temperatures, conductivity bands are empty.

Notwithstanding the fundamental objections to energy migration in native proteins, Blyumenfel'd (1957) considered the participation of triplet states in energy transfer, a possibility that is partially confirmed by the fact that the phosphorescence spectrum of proteins lies in the region of 4000 A and corresponds closely to the calculated excitation energy which would be sufficient for energy transfer across molecular orbitals originating within the framework of hydrogen bonds. However, in the absence of prosthetic groups, it seems reasonable that few, if any, electrons would be free for mobilization. When prosthetic groups are present, however, and when they lie close to those of the protein (energetically speaking), electrons could be transferred into low lying orbitals of protein. Once in these molecular orbitals, the forbidden triplet transition to the singlet ground state would tend to maintain electrons in the triplet state energy levels. Under these circumstances, the electron would move along a chain of peptide hydrogen bonds until disposed of into some other favorably bound group.

RADICAL CONTENT IN PROCESSED FISHERY MATERIALS

For conventionally processed foodstuffs, tissues, and meals (that is, solvent-extracted proteins or proteins processed at room temperature), the situation is quite different. No longer do we have a protein substance identical in character to the native material; bonds have been broken, and the original geometrical arrangement of the protein chains has been completely disrupted. In many instances, certain compounds or classes of compounds have been selectively removed while at other times other substances are purposely added back to the protein.

Interestingly, the present study has shown that such materials as protein concentrates containing some residual lipid, fish meal, and freeze-dried tissue samples not processed with utmost caution in the freezing and freeze-drying steps, all exhibit characteristic EPR signals when exposed to air. Indeed, it is generally true that the radical content of haphazardly handled materials is usually higher than for an equal weight of similar material which has been processed by careful handling, freezing in liquid nitrogen, and careful removal of water. Unable to migrate along conductive pathways, effective charge transfer to radicals from donor molecules or reactants is apparently reduced (thus the radicals act as though caged or matrixed) and no longer do radicals interact freely with one another nor do they react at once with other cellular constituents. However, the efficiency in the reduction of charge transfer undoubtedly depends on the nature of the sample and its treatment. Just how immobilized such radicals are is open to conjecture. They are im-

mobilized sufficiently, however, so as to be detectable for fairly long periods of time.

CHARACTERISTIC FORM AND APPEARANCE OF EPR SIGNALS OBSERVED IN DRIED PRODUCTS

Although a high resolution EPR analysis can usually be performed with dilute solutions of soluble, low molecular weight organic radicals, the same is seldom true for powdered samples, and especially for powdered samples of complex molecules. The requirements for resolution are: magnetically dilute systems (in order to prevent spin-spin interaction), long relaxation times, and a low rf field. In the solid state, relaxation times are shortened because of the more effective coupling between spin states and the surrounding lattice—cooling the sample (perhaps to the temperature of liquid nitrogen or below) will often increase the relaxation time to acceptable values. Related to relaxation is the line broadening arising with molecular dipole interactions. The notable example is the so-called "oxygen effect"—some radicals will be far removed from the magnetic influences of the molecular oxygen di-radical while other free radicals in the sample will be near oxygen molecules. Consequently, free radicals of the sample will experience a variety of magnetic fields, producing a collective band of resonances resulting from the distribution of collective magnetic fields superimposed on the external instrument magnetic field. Therefore, in solid state studies, radicals, because of their random alignment, exhibit anisotropic coupling which broadens the lines and makes interpretation difficult. Spectral features which can be used to characterize the radicals are the measurement of the g-value, line shape, and changes in these parameters upon chemical or physical treatment of samples.

FIGURE 1.—EPR spectra for protein essentially free of lipid and for a lipid-treated protein, all exposed to air. A, Freeze-dried and solvent extracted rockfish myofibrillar protein. B, Freeze-dried human serum albumin. C, Crude bovine serum albumin (BSA) (upper trace). Crude BSA + C22:6 fatty acid (2:1 by wt) oxidized in air at room temperature for 2 hr (middle trace). Same material in air at room temperature at the end of 4 hr (lower trace). The arrows denote the g = 2 or free-spin value.

FIGURE 2.—EPR lipid signals in marine protein concentrates exposed to air. A, Freeze-dried rockfish flesh exposed to air for 20 hr at room temperature. B, Commercially available FPC, now 2 years old, low lipid initially, which still exhibits a weak lipid signal. C, Freeze-dried silver salmon light flesh exposed to air for 10 hr at room temperature. Arrows denote g = 2.

EPR SPECTRA OF FISHERY PRODUCTS

Shown in Figures 1, 2, and 3 are EPR signals which are observed in lipid-protein models and in dry ice-frozen, freeze-dried fish tissue, all of which are under investigation in this laboratory. As with carefully freeze-dried samples of the type discussed above (liquid nitrogen frozen and freeze-dried), the resonances are devoid of hyperfine structure of the type normally

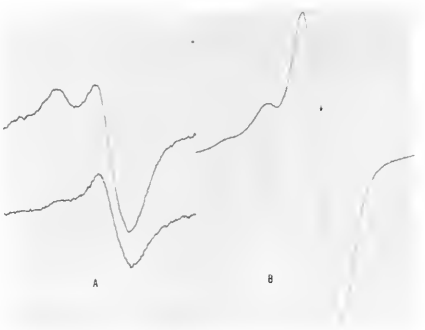


FIGURE 3.—EPR lipid signals in a hydroperoxide-treated protein and in a sacroplasmic protein. A. Rockfish myofibrillar protein upon removal from the freeze-drier (lower trace). The same material 40 min after incorporating a small amount of a hydroperoxide mixture prepared from a marine oil (upper trace). B. Freeze-dried rockfish sacroplasmic protein after storage in air at room temperature for 1 day. Arrows denote g = 2.

observed for dilute solutions of low molecular weight radicals. Without exception, all powdered materials of proteinaceous nature, which exhibited any type of signal at all, gave a single absorption line in the "free-spin" or g = 2 region. The g = 2 signal is exemplified in Figure 1a (for solvent-extracted myofibrillar protein), or in Figure 1b for human serum albumin. I will have more to say about the g = 2 signal; however, let us concern ourselves for the moment with other resonances which are seen in those samples containing oxidizable lipid in addition to protein.

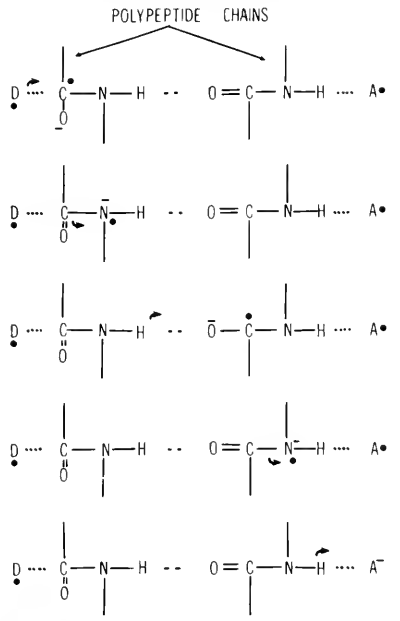
Freeze-dried Pacific cod, silver salmon, rockfish, and other marine fish, though devoid of the g = 2 signal initially (before lipid oxidation has taken place) soon give rise to two resonances when samples are exposed to air—the central g = 2 resonance and, downfield (to the left) from the central resonance, an area of EPR activity which I have designated as the "lipid signal" region (Figures 2 and 3). Unlike tissue samples, many single proteins considered to be quite pure exhibit a g = 2 resonance only. When, however, a thin film of oxidizable lipid is deposited on such materials and the mixture is exposed to air, in addition to the central resonance, a lipid signal is also observed (Figure 1). A preliminary study of lipid signals in various models is to be found in the recent work of the author (1970). Although it has not been possible to measure the g-value with the necessary precision needed to fingerprint the radical completely, the available data suggest a radical of the peroxy type. This is further illustrated by the two traces of Figure 3a. No indications of hyperfine splitting (hfs) are also consistent with a radical of this nature.

CHARGE TRANSFER IN TISSUES

In the present study, it is of particular interest to find that in carefully handled freeze-dried tissue samples, there often occurs after the lipid signal reaches a maximum, an abrupt increase in the g = 2 region. In those "native" samples containing a complement of cellular lipid, this may indicate a charge migration (a strong D→A—donor-to-acceptor interaction; strong charge-transfer process) between a cellular constituent acting as a donor and a peroxy radical acceptor. Such a process is also consistent with the observation that it is at this point in time that the lipid signal begins to decay. This draws our attention to the likelihood that once radical content has increased to some critical concentration, overlap of wave functions between a radical acceptor and a donor is sufficient to allow reactions to proceed. The abrupt change in the g = 2 region is illustrated by the spectra of Figure 3. Figure 3b for sacroplasmic protein under air for 1 day is to be compared with the lower trace of Figure 3a for the same material immediately on removal from the freeze-dryer.

Another point in favor of a mechanism of this type is the fact that only proteins are really effective as matrices for the formation as well as for the decay of radicals. Powdered glass, quartz wool, and amino acids are essentially without effect when used as substrates for thin films of reactants. Although there are many unanswered questions concerning the mechanism

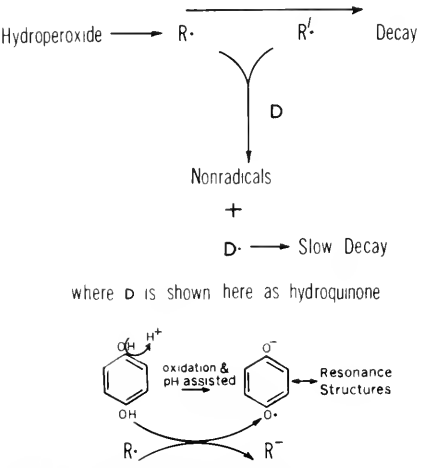
of trapping and charge migration, the data are consistent with the scheme shown below:



SCHEME 1.—Charge-transfer between donor (D) and a free radical acceptor (A) in biological systems. Although the actual pathway is not known with certainty, available data are in accord with the idea that hydrogen bonding of the type shown may play a role in the transfer of charge.

where D is a cellular electron donor material acting in the role of an antioxidant and A is a peroxy radical acceptor. To completely substantiate that such a mechanism does exist would be very exciting indeed for this would be the first instance in which the role of an antioxidant at the molecular level could be designated as a strong D→A interaction.

The data are also in accord with the idea put forth sometime ago by some Russian investigators, that the g = 2 signal in tissue is semiquinone in nature (Chetverikov, Blyumenfel'd, and Fomin, 1965). In the present work, for instance, when hydroquinone or various hydroquinone derivatives with free hydroxyl groups are incorporated into proteins coated with thin films of unsaturated lipid, an enhanced central resonance is obtained which is identical to that obtained for oxidizing tissue alone. For the protein-lipid hydroquinone systems, the author has shown that central resonance consists chiefly of trapped semiquinone radical ions. The g = 2 resonance in "lipid-free" proteins may very well indicate prior lipid oxidation but now at a point in time at which lipid signal has decayed. The g = 2 retention could then be explained because of resonance stability or other stabilizing factors for this species. For instance, some commercial preparations of bovine serum albumin exhibit an EPR signal somewhat displaced from the usual g = 2 signal while others do not. Likewise some of the samples that are EPR-active exhibit fluorescence quite characteristic of malonaldehyde-amino acid interaction (formation of iminopropene derivatives). The various data taken collectively suggest the following mechanism:



SCHEME 2.—Interaction between radicals (R·, R·') and cellular antioxidants (D).

Other radicals, however, may make some small contribution to the central resonance pattern. A recent investigation by Wekell and Roubal[2] has shown that free radicals arise during carbonyl amine browning. What is more, although hfs is seen in early stages of the browning, the signal changes to a single line in the $g = 2$ region as polymerization progresses. The browning reaction, without the implication of free radicals in lipid-protein systems, though not a dominant pathway to pigments, was first discussed by Venolia and Tappel (1958) as a possible cause of color formation during the oxidation of such systems [but because the more recent work, polymeric masses are, for the most part, attributed to lipid peroxy induced protein-protein polymerization together with malonaldehyde cross-linked proteins (conjugated Schiff base)].

Thus, although recent studies in this laboratory have uncovered new facts concerning radicals in lipid-protein systems, the exact nature of the resonances observed in freeze-dried tissue and in dry model mixtures remains to be fully characterized. Nonetheless, this pioneering piece of research has paved the way for use of EPR studies in systems of oxidizing lipids together with other cellular constituents. Concurrent studies have shown that transition metal ion impurities, if present, play only a minor role in radical production. Other studies of this laboratory have shown that protein-free fish bone does not give EPR signals. Freshly prepared freeze-dried tissue samples give no lipid signal resonances, but signal amplitudes grow on exposure to oxygen. Depending on the type of protein, type and amount of lipid, or added material, lipid signals exhibit various lifetimes ranging from hours to years. (For instance, compare Figure 1c for BSA with Figure 2b for 2-year-old FPC.) Polysaccharides are only partially effective as radical matrices.

For living systems, or for any system containing residual and unprotected oxidizable lipid, the implications of the various interactions discussed are significant. It is known that products of lipid oxidation in lipid-protein systems interact with proteins, enzymes, and nucleotides. Not only are these native biopolymers further polymerized by such interactions, constituent building blocks are destroyed (Roubal and Tappel, 1966b, 1967); notable are the sulfur amino acids which have been shown to be easily destroyed by free radicals (Roubal and Tappel, 1966a). In this presentation I have not discussed consequences of unwanted lipid peroxidation in nutritional deficiency states or in other pathologies in living systems. Nevertheless, such lipid-protein interaction is quite significant. The geronotological implications of these reactions leading to the formation of age pigments, based on studies of Roubal and Tappel (1966b) and others, have been reviewed by Bjorksten (1965), Packer, Deamer, and Heath (1967), and Tappel (1968).

[2] Unpublished data; to be published.

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THE RELATION BETWEEN EXERCISE AND BIOCHEMICAL CHANGES IN RED AND WHITE MUSCLE AND LIVER IN THE JACK MACKEREL, *Trachurus symmetricus*

AUSTIN W. PRITCHARD,[1] JOHN R. HUNTER,[2] AND REUBEN LASKER[2]

ABSTRACT

Glycogen, lactic acid, and fat concentration in red and white muscle and glycogen in the liver of jack mackerel, *Trachurus symmetricus*, were measured after periods of forced swimming by *Trachurus* at speeds above, below, and at the sustained speed threshold. Failure to swim at any speed was associated with an almost complete depletion of glycogen in the white muscle only. The trend of glycogen use in the red muscle closely followed that of the liver and was not correlated with failure to swim. Reduction of glycogen levels in red muscle and liver were associated with extended periods of swim- Lipid use was slow and not correlated with fatigured muscle and was insignificant in white muscle. ming. High lipid content was characteristic of e. A decline in lipid concentration after exercise occurred only in the red muscle and only after a swimming period of 6 hr at a subthreshold speed. High lactate levels were characteristic of both muscle types and did not appear to be related to fatigue at any swimming speed.

The high lactate levels in white muscle, the almost complete depletion of glycogen in the white muscle of exhausted fish, and the parallel pattern of glycogen depletion in red muscle and liver suggested that white muscle was the primary locomotor organ near and above the threshold for sustained speed. At these speeds red muscle like the liver may provide nutrients to the white muscle, provided time for mobilization is sufficient. At speeds below the sustained speed threshold our analysis indicated that both the red and white muscle systems were used but the relative significance of the locomotory role played by each system could not be evaluated.

The lateral musculature of many fishes may be readily segregated by color into red and white portions. Typically in active fishes the red muscle makes up from 10 to 20% of the total musculature and is arranged in a thin lateral sheet just beneath the skin whereas the white muscle makes up the underlying mass of the myotome. The two muscle types also differ in the diameter of their muscle fibers, speed of contraction, blood supply, mitochondrial content, patterns of innervation, and glycogen and fat content (Bone, 1966).

The accepted view of the function of red and white muscle tissues in fishes was outlined by Bone (1966). He concluded from his own work on dogfish and from an extensive literature review that the two muscle fibers represent two separate motor systems which operate independently, utilize different metabolites, and serve different locomotory functions, viz., the red muscle is used for slow cruising speeds and functions by aerobic metabolism of fat whereas the white muscle is used for rapid bursts of swimming and is driven by anaerobic glycolysis. Bone's conclusions have subsequently been supported by measurements of oxygen uptake in red and white muscle by Gordon (1968) and by electrophysiological studies on oceanic skipjack, *Katsuwonus pelamis*, by Rayner and Keenan (1967). On the other hand, Braekkan (1956) and Wittenberger (1967) believe the red muscle has no independent locomotor role and functions as a metabolic organ for the white muscle. Electrode recordings from the red muscle (Bone, 1966; Rayner and Keenan, 1967) have provided irrefutable evidence for an independent locomotor function of red muscle at certain slow speeds, but the metabolic independence of the two muscle systems and their metabolic and locomotor function at higher speeds is still open

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to question. Although the roles assigned to the two muscle systems are dependent on swimming speed, no studies have been made on the function of the muscle systems using normally swimming intact animals at known speeds. The objective of this study was to re-examine the metabolic and locomotor roles of red and white muscle by measurement of glycogen, lactate, and fat levels in the muscle and glycogen levels in the liver in fish exposed to various velocity treatments of known strength and duration. Juvenile jack mackerel, *Trachurus symmetricus*, were used in this study because the maximum sustained speed threshold for 6 hr of continuous swimming had already been established for this species (Hunter, 1971), and consequently we were able to relate all of our chemical measurements to known levels of swimming performance.

METHODS AND PROCEDURES

SWIMMING TESTS

Jack mackerel were maintained at a regulated seawater temperature of 18.5° C in a plastic swimming pool 4.57 m diameter and fed an abundant ration of brine shrimp, *Artemia*, and chopped fish and squid each day. The fish were not fed for 20 hr prior to testing. Jack mackerel were tested in an activity chamber patterned after that of Beamish (1968) and described in detail by Hunter and Zweifel (1971). The swimming compartment of the apparatus consisted of a tube 230 cm long and 41 cm in diameter through which seawater could be moved at speeds ranging from 12 to 212 cm/sec. Fish were placed in the tube and forced to swim at a water speed for certain periods varying from 8 min to 6 hr. At the end of the swimming period they were removed and dropped immediately into liquid nitrogen and the frozen fish were stored at —30° C until used for chemical analysis. The time required for removal and freezing did not exceed 1 min.

Speed treatments for the experiments were chosen relative to the 50% endurance threshold for jack mackerel at 22 $L^{0.6}$/sec for 6 hr of swimming where L is total length (Hunter, 1971). Five jack mackerel, mean length 14.6 cm, were tested at the subthreshold speed of 19.6 $L^{0.6}$/sec (98 cm/sec); 14 jack mackerel, mean length 16.3 cm, were tested at the near threshold speed of 21.1 $L^{0.6}$/sec (113 cm/sec); and 10 jack mackerel, mean length 14.7 cm, were tested at the superthreshold speed of 27.7 $L^{0.6}$/sec (139 cm/sec). Fish tested at the subthreshold speed swam continuously for 6 hr and were sampled at the end of that period. Fish tested at the threshold speed were divided into two groups: seven fish that were sampled after 6 hr of continuous swimming; and seven fish that fell from exhaustion at some time during the 6-hr period. The latter group of seven fish were quickly removed from the apparatus and frozen as soon as they fell against the rear screen. Fish tested at the superthreshold speed were also divided into two groups: those that swam successfully for 8 min; and those that failed after 8 or less minutes of swimming.

Ten jack mackerel, mean length 14.5 cm, were used as controls. Five of the control animals were removed from the holding tank, placed in the apparatus, allowed to swim for 30 min at the slow speed of 6.2 $L^{0.6}$/sec (30 cm/sec), removed, and frozen. The other five control fish were removed from the holding tank and immediately frozen. The data from these two control groups were later combined because no difference between them was detected.

CHEMICAL ANALYSES

White and red muscle were dissected from the frozen fish while still frozen. One lateral strip of red muscle was used for fat analysis and the other divided into two equal portions for lactate and glycogen analysis respectively. About 1 g of white muscle from the dorsal portion of the myotome was used for glycogen determinations, 0.5 g for lactate, and 0.5 g for fat measurements. Fish were returned to the freezer and liver samples (0.1-0.2 g) were analyzed for glycogen about a month after the muscle determinations.

For lactate measurements muscle was quickly cut into small pieces, weighed, and homogenized in 10% trichloroacetic acid in prechilled tubes. Proteins and cellular debris were spun down in a clinical centrifuge. Aliquots of the protein-free supernatant fluid were analyzed for lactate enzymatically using the test reagents supplied by SIGMA Chemical Company.[3] The test is based on the conversion of nicotine adenine nucleotide (NAD) to the reduced form (NADH) as lactate is converted to pyruvate by lactate dehydrogenase. All readings were made at 340 mμ on a Beckman DU spectrophotometer. Results are expressed as mg of lactic acid per 100 g wet weight muscle tissue.

Muscle and liver samples for glycogen determinations were dropped into preweighed graduated centrifuge tubes containing 3 ml of 30% potassium hydroxide. Glycogen was precipitated with alcohol and determined according to the method of Montgomery (1957). All readings were made at 490 mμ on a Beckman DU spectrophotometer. Results are expressed as mg glycogen (as glucose) per 100 g wet weight in the case of muscle, and as percent glycogen in the case of liver.

Muscle tissue was dried in an oven at 60° C to constant weight for fat analysis. Fat was removed by a soxhlet extraction with chloroform-methanol (2:1, v:v). After the extraction the solvent in the tissue was evaporated and the difference in weight of the tissue recorded (Krvarić and Mužinić, 1950).

[3] P.O. Box 14508, St. Louis, Mo. 63178. Reference to commercial products does not imply endorsement.

RESULTS

Fish that swam continuously for 6 hr at the subthreshold speed of 98 cm/sec and at the threshold speed showed no difference in the glycogen content of the white muscle from the controls (Table 1). On the other hand, in fish

TABLE 1.—Glycogen in red and white muscle, and liver of jack mackerel following various forced swimming conditions. Red and white muscle glycogen in mg per 100 g wet weight; liver glycogen is percent of wet weight. -- indicates measurement was lost during analysis.

	Controls			19 $L^{0.6}$ Subthreshold speed			21 $L^{0.6}$ Threshold speed successes		
	Red	White	Liver	Red	White	Liver	Red	White	Liver
	76.6	85.9	6 42	15 23	53.90	0 125	26.71	204 3	0.043
	102.8	--	9 49	52.80	143.2	4.36	33.33	159.4	1.63
	176.3	--	22.74	192.5	76.69	.317	37.59	80.74	1.85
	277.8	276.8	8.75	145 2	316.2	8.31	95 93	492 8	.125
	562 0	142.6	18.59	147.6	102.6	3.16	152 4	223 2	3.42
	706.0	157 9	18.17				191.7	638 0	3.19
	1075	267.8	10.26				475.1	216.3	1.38
	1394	72 9	17.00						
	1417	71.1	11.82						
	1706	216 5	24.24						
Mean	749.4	161.4	14.75	[1]110.7	138 5	[1]3.25	[1]144.7	287.8	[1]1.66

	21 $L^{0.6}$ Threshold speed fatigued			28 $L^{0.6}$ Superthreshold speed-individually fatigued			28 $L^{0.6}$ Superthreshold speed, 8-min test		
	Red	White	Liver	Red	White	Liver	Red	White	Liver
	11.5	26 50	078	--	--	0.546	--	39 93	10.00
	16.00	4.51	.034	149.6	8.02	4.73	298.6	141 9	10 00
	17.10	20.63	3.30	215.0	19.56	3.83	473.3	25 44	3.16
	40 55	27.77	5.04	490 4	6.76	13 23	533.1	141.0	10 94
	104.8	--	11.96	654 5	18.49	5 81	553.9	57.22	11.31
	151.8	11.15	.820						
	241.5	50.2	12.50						
Mean	[1]83 27	[1]23.46	[1]4.82	377 4	[1]13.21	[1]5 63	464.7	[1]81 1	9 08

[1] Differed from the controls, $P \leq 0.05$, Mann Whitney U test (Siegel, 1956).

that failed to swim the full 6 hr at the same speed the glycogen levels in the white muscle were lower and were different from the controls ($P = 0.001$ Mann Whitney U test, Siegel, 1956). Glycogen levels in white muscle of all fish tested at the superthreshold velocity were also much lower and statistically different from the controls ($P = 0.05$). The lowest glycogen levels of all were in fish that failed from exhaustion at superthreshold speeds. The values in these exhausted fish were statistically different from those of fish that swam at the same speed but which were removed after 8 min of swimming before they could fall from exhaustion. In sum, strenuous exercise and exhaustion regardless of speed were associated with a marked depletion of glycogen reserves in the white muscle, whereas successful swimming for 6 hr at subthreshold or threshold speed produced no significant change in white muscle glycogen.

The glycogen content of the liver and red muscle were lower and different from the controls in fish tested at threshold and subthreshold speeds ($P = 0.05$). At superthreshold speed, on the other hand, the glycogen content of the red muscle was not different from the controls and that of the liver was different only in fish that failed from exhaustion ($P = 0.02$).

Thus, the trends in the levels of red muscle and liver glycogen in relation to swimming speed were nearly the reverse of that for white muscle glycogen. Low levels of glycogen in red muscle and liver were associated with slow speeds that could be sustained for extended periods. These results suggest that glycogen from red muscle and liver provide energy to the white muscle at nearly all swimming speeds. We believe that no drop occurred in red muscle glycogen in fish fatigued at high speeds because the time was too short for the white muscle to mobilize significant amounts of glycogen. This view is supported by the negative correlation between the level of glycogen in the red muscle and swimming time to fatigue at threshold speed. This is illustrated in the following table:

Threshold speed $= 21\ L^{0.6}$

Time to fatigue (*min*)	Glycogen in red muscle (*mg per 100 g wet weight*)
282	11.5
110	16.0
131	17.1
79	40.6
15	104.
38	241.

($r_s = -0.857$, $P < 0.05$)

In fish exercised at the superthreshold speed the lactic acid content of the red and white muscle was considerably above that of the controls and statistically different from them ($P = 0.05$) (Table 2). At threshold and subthreshold speeds, the lactic acid concentration in red and white muscle formed no distinct pattern. At threshold speed the lactate levels of red and white muscle were about the same as the controls and did not differ from them except for one case where the values were actually lower

TABLE 2.—Concentration of lactic acid in red and white muscle of jack mackerel following various forced swimming conditions. Values given are mg lactic acid per 100 g wet weight.

Controls		19 $L^{0.6}$ Subthreshold speed		21 $L^{0.6}$ Threshold speed successes	
Red	White	Red	White	Red	White
40.49	233.0	94.6	520.9	20.60	310.4
59.29	425.3	97.6	596.6	22.83	344.5
71.58	387.5	99.2	521.9	26.79	385.3
77.46	521.0	117.1	630.3	45.66	464.8
79.53	570.3	156.8	762.3	56.94	403.1
82.19	341.5			82.19	345.2
86.76	390.6			83.87	410.2
86.76	319.0				
86.76	553.4				
95.44	589.6				
Mean 76.63	433.1	[1]113.1	606.4	[1]48.41	380.5

21 $L^{0.6}$ Threshold speed fatigued		28 $L^{0.6}$ Superthreshold speed - individually fatigued		28 $L^{0.6}$ Superthreshold speed, 8-min test	
Red	White	Red	White	Red	White
26.23	564.6	101.2	422.9	124.2	724.1
39.61	545.3	108.4	538.6	132.3	668.9
56.58	404.4	120.4	723.1	189.0	799.9
66.6	499.2	151.9	745.8	202.8	807.4
86.76	489.7	230.5	800.6	237.4	733.5
122.5	486.2				
205.5	646.1				
Mean 86.25	519.4	[1]142.5	[1]646.2	[1]177.1	[1]746.8

[1] Differed from the controls, $P \leq 0.05$, Mann Whitney U test (Siegel, 1956).

than the controls; at this subthreshold speed lactate levels of red and white muscle were higher than the controls and differed statistically ($P = 0.02$). We have no explanation for these differences except to suggest that the high muscle lactate concentration in the control animals may have obscured changes resulting from moderate exercise. A larger sample size may be required to obtain reliable measurements of differences in lactic acid concentration caused by moderate exercise.

Muscle lactate level did not appear to be related to fatigue at any swimming speed. Lactate levels in fish that fatigued at the threshold speed were not different from the controls. Fish that failed at superthreshold speeds had a higher muscle lactate level than did the controls but the level did not differ from that of fish that swam at the same speed but were removed before they became exhausted. These results suggest that high lactic acid concentration in muscle was not the principal cause of exhaustion.

Red muscle contained considerably more fat per unit weight than white muscle. Indeed, white muscle fat levels were almost undetectable in many cases (Table 3). White muscle fat levels did not differ from the control at any speed level. Red muscle fat did not differ from the controls at threshold and superthreshold speeds but at the subthreshold speed the mean level of fat in the red muscle was lower than the controls and differed statistically from them ($P = 0.02$). Thus only when the fish swam for at least 6 hr at subthreshold speed was there evidence of fat utilization in the red muscle.[4] The reduction in fat in the red muscle suggests that the red muscle system may have been used at the subthreshold velocity. On the other hand, presence of high muscle lactate in both red and white muscle and the drop in red muscle and liver glycogen at subthreshold speeds implies that the white muscle was also active.

TABLE 3.—Fat analyses in red and white muscle of jack mackerel following various forced swimming conditions. Where 0.0% is given for white muscle, only traces of fat were found with the chloroform-methanol extraction. For convenience zeros were used for averaging. Values given as percent dry weight of tissue.

Controls		19 $L^{0.6}$ Subthreshold speed		21 $L^{0.6}$ Threshold speed successes	
Red	White	Red	White	Red	White
20.16	1.98	15.07	0.230	16.91	0.0
21.00	0.0	15.69	2.12	22.45	0.0
21.78	.337	16.19	1.24	23.80	2.19
22.24	1.15	20.54	0.0	24.67	0.0
23.05	1.32	24.63	0.0	24.97	1.54
25.11	0.0			26.43	4.09
25.14	.390			29.37	4.10
25.89	.924				
27.29	2.65				
32.32	2.20				
Mean 24.40	1.10	[1]18.42	.718	24.08	1.70

21 $L^{0.6}$ Threshold speed fatigued		28 $L^{0.6}$ Superthreshold speed - individually fatigued		28 $L^{0.6}$ Superthreshold speed, 8-min test	
Red	White	Red	White	Red	White
21.30	0.0	16.54	0.0	21.96	2.01
21.97	0.0	16.71	0.0	23.08	.04
22.47	0.0	26.47	1.22	24.20	.18
28.70	0.0	28.19	.732	25.57	.50
30.11	2.56	28.70	2.13	29.54	2.26
32.08	1.12				
32.90	6.46				
Mean 27.08	1.45	23.32	.816	24.87	.998

[1] Differed from the controls, $P = 0.02$, Mann Whitney U test (Siegel, 1956).

DISCUSSION

Control levels of jack mackerel white muscle glycogen were similar to those recorded by Canadian workers for mixed red and white muscle in salmonids (Black, Robertson, and Parker, 1961; Black et al., 1962; Connor et al., 1964) and to those from a variety of marine teleosts (Beamish, 1968; Fraser et al., 1966; Wittenberger, 1968; Wittenberger et al., 1969). Red muscle glycogen has not often been separately determined. Our mean control value of 750 mg percent was somewhat higher than the mean of 420 mg percent reported by Wittenberger (1968) for *Trachurus mediterraneus ponticus*, a related species from the Black Sea. Fraser et al. (1966) gave a range of 215 to 279 mg percent for red muscle glycogen of cod, based on analysis of three fish in a relaxed (anesthesized) state. Wittenberger et al. (1969) reported 320

[4] In an earlier and preliminary experiment, five smaller jack mackerel, mean length 9.2 cm, swam at the subthreshold speed of 12.7 $L^{0.6}$/sec (48 cm/sec) for 48 hr without failure and we recorded a decrease in the mean fat content of red muscle from 23.7% (range, 20.4-28.4%; $n = 5$) to 18.0% (range, 16.2-20.8%; $n = 5$) ($P<0.05$).

mg percent in a clupeid, *Harengula humeralis*. A much higher level of 1866 mg percent was given by Bone (1966) for dogfish. In most cases, the concentration of glycogen in red muscle was considerably higher than in white muscle.

Liver glycogen controls in jack mackerel were much higher than those reported previously in teleosts. Connor et al. (1964) for example, obtained values of about 1% in chinook and sockeye salmon and steelhead trout, and found that moderate exercise associated with ascending fishways had no effect on liver glycogen levels. Black et al. (1960) reported liver glycogen levels of 0.5-4% in rainbow trout, and Dean and Goodnight (1964) obtained 0.8-3% in four species of warmwater centrachid fishes. Values similar to ours were reported by Wittenberger and Diaciuc (1965) in carp (13.8%) and by Bellamy (1968) in recently fed red piranha (10.3%). Even if a high degree of gluconeogenesis were operative in jack mackerel, it seems unlikely that this could entirely explain the high levels of liver glycogen.

Control levels of glycogen in jack mackerel white muscle appeared to be similar to those in other fishes. However, in the red muscle and especially in the liver, glycogen levels were usually higher than in fishes studied earlier.

The most striking finding of this study was the virtually complete depletion of glycogen in the white muscle of fish that failed from exhaustion. The depletion of glycogen in white muscle occurred in all fish that failed regardless of the speed of swimming or how long they swam. In fish that did not fail at a near threshold speed of 21 $L^{0.6}$/sec (Hunter 1971) the glycogen in the white muscle did not differ from controls, whereas in the fish that failed, glycogen in the white muscle was at nearly the same low level as it was in fish that failed after a few minutes of exertion at a much higher speed. Red muscle glycogen was also depleted at some swimming speeds but the pattern of glycogen depletion in red muscle closely paralleled that of the liver. Red muscle had one-fifth the lactate found in white muscle on a percent basis but only about one-fiftieth on an absolute basis because the mass of white muscle exceeds the red by 10 to 1.

The high lactate levels in the white muscle, the almost complete depletion of glycogen in the white muscle of exhausted fish, and the parallel pattern of glycogen depletion in red muscle and liver all point to the same hypothesis. In jack mackerel at threshold and higher speeds the energy used for swimming was derived primarily from glycolysis in the white muscle which was the principal locomotor organ. Red muscle like the liver may serve as a storage organ whose resources could be used to drive the white muscle, given sufficient time for mobilization. Thus at threshold speeds, red muscle function appeared to be tied to that of the white and it could not be considered as acting independently. No change in red muscle glycogen was detected at the highest test speed, possibly because time was insufficient to mobilize the glycogen reserves other than in the white muscle itself. This time dependency for mobilizing red muscle glycogen under conditions of strenuous exercise could explain why Bone (1966), Wittenberger and Diacuic (1965), Wittenberger (1968), and Fraser et al. (1966) detected no change in red muscle glycogen after strenuous exercise. It must be remembered that in all of these previous studies the strength and the duration of the exercise was unknown, except that it was considered to be extreme.

The decrease in fat content plus the high lactate levels suggest that the red muscle was used for swimming at subthreshold speeds. Bilinski (1969) showed that the rate of oxidation of fatty acids in red muscle of rainbow trout and sockeye salmon exceeded that in the white muscle by one or more orders of magnitude depending on the fatty acid substrate. On the other hand, neither the high oxidative capacity nor the decline in lipid levels in red muscle with moderate exercise are sufficient evidence for an independent locomotor role. In addition, the presence of high lactate levels in white muscle and the drop in the glycogen content of the white muscle indicated that the white muscle was also used at the subthreshold speed of 19 $L^{0.6}$/sec. The electrophysiological evidence for indepen-

dent locomotor activity of the red muscle cannot be ignored. At some speed slower than any used in the present experiment jack mackerel may depend only on red muscle for propulsion and on lipids for fuel. At what velocity red muscle begins to play a major role or how significant this speed may be in the life of the animal are questions that remain to be answered. The most tenable explanation for these data is that both muscle systems were used at the subthreshold speed but we are unable to choose which system played the more significant role.

Jack mackerel appear to be specialized in body form and swimming capabilities for high-speed continuous swimming (Hunter, 1971). Thus the physiological characteristics we have described, namely use of glycolysis in white muscle for swimming, high liver glycogen levels, and tolerance of high muscle lactate levels may represent specializations for high-speed swimming and may not be representative of the general pattern in fishes. On the other hand, *Trachurus* may share these characteristics with other fishes of similar habits, for example other carangids and the scombroid fishes. It seems possible that evolution may have favored the development of these physiological characteristics because severe velocity limits may be set by aerobic lipid metabolism.

ACKNOWLEDGMENTS

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Sebastes variegatus, SP. N. FROM THE NORTHEASTERN PACIFIC OCEAN (PISCES, SCORPAENIDAE)

JAY C. QUAST[1]

ABSTRACT

A new scorpaenid fish, *Sebastes variegatus*, from the Gulf of Alaska is characterized by an elongate body that tapers symmetrically anteriorly and posteriorly; presence of preocular, postocular, tympannic, and parietal spines and lack of supraocular, coronal, and (usually) nuchal spines; 18 (rarely 17 or 19) rays in the pectoral fin; a second anal fin spine that is longer than the third; black membranes in the spinous dorsal and caudal fins; a dark brown to jet black peritoneum; and a dark blotched pattern on the sides that is interrupted over the posterior 2/3 of the body by an unpigmented band along the lateral line. The known geographic range is from Unimak Pass (Aleutian Islands) to Queen Charlotte Sound (British Columbia).

On February 28, 1967, the Bureau of Commercial Fisheries RV *Murre II* obtained three specimens of a new species of rockfish from the vicinity of Point McCartney in Frederick Sound, southeastern Alaska. The specimens were captured when a 6-ft-diameter Isaacs-Kidd trawl inadvertently was allowed to touch bottom at 135 m.

The specimens were tentatively identified as *Sebastes zacentrus* but were not adequately described by Phillips' (1957) account of this species. In some respects they seemed to be intermediate between *S. zacentrus* and *S. proriger*. The evidence for an undescribed species became convincing when additional specimens were taken, and the similarity to *S. zacentrus* and *S. proriger* was underscored when several more specimens were found in old collections that had been labeled as these species.

The species is placed in *Sebastes* Cuvier in concurrence with opinions of numerous authors that northeastern Pacific *Sebastodes* are congeneric with North Atlantic *Sebastes*—including Matsubara (1943b:178); Tsuyuki, Roberts, Lowes, Hadaway, and Westrheim (1968:2494); Eschmeyer (1969:104); Chen (1969:12); and American Fisheries Society (personal communication with Reeve M. Bailey, University of Michigan, December 1969).

The name *variegatus* refers to the contrasting coloration of most specimens when fresh. According to Brown (1956:830), the word means "of different sorts, particularly colors." I suggest "harlequin rockfish" as the common name.

MATERIALS AND METHODS

The study utilized 39 specimens of *S. variegatus* and included 35 from the ichthyological collection of the National Marine Fisheries Service Biological Laboratory at Auke Bay, Alaska, and 3 from the collection at the Institute of Animal Resource Ecology, University of British Columbia, Vancouver, Canada. This series included 21 males, 13 females, and 4 of unknown sex, and no important differences were found between the sexes in measurements, counts, or coloration. Measurements on the University of British Columbia specimens were not as complete as those from the Auke Bay Laboratory; hence the numerical basis of the morphometric analysis varies by 3, depending on the character. An additional male specimen was used for electrophoretic analysis.

Methods for counts and measurements are based on Hubbs and Lagler (1949:8-15), with the following exceptions or modifications. In counts: tubed lateral line scales on caudal fin lie after hypurals; tubed scales over hypurals are included in count of tubed lateral line scales

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on body; and scale rows are taken below lateral line. In measurements: standard length (SL) is measured from anterior upper lip or upper teeth, whichever is more anterior, to caudal base; head length is measured from midline on upper jaw to limit of opercular flap; interorbital width equals bony interorbital measurement; lower jaw projection equals distance that lower jaw extends anteriorly to upper jaw, projected on longitudinal axis of fish; length of gill raker equals length of raker at bend of gill arch, measured from fleshy tip to, but not including, T-shaped base; length of prenarial pore equals length of enlarged pore on anterior snout near junction of premaxilla and premaxillary process; length of subnarial pore equals length of large pore on a shelf formed by upper margin of lacrimal bone, immediately below nares; body depth (pelvic) is measured from articulation of pelvic fin spine; body depth (anal) is measured from base of anal fin anterior to first anal spine; length of pectoral fin is measured from base of uppermost ray to apex of fin when fin is aligned with longitudinal axis of fish; length of soft dorsal fin base is measured from axil of last dorsal fin spine to axil of last soft ray; length of base of anal fin is measured to axil of last ray; and length of pelvic fin is measured from articulation of pelvic fin spine to tip of fin.

Terminology for spines and ridges of head follows Jordan and Evermann (1898, II:1765, 1768).

Sebastes variegatus SP. N.

(FIG. 1-3)

HOLOTYPE

(Original number AB[2] 69-25); 201 mm SL; male; Gulf of Alaska south of Kodiak Island (approximately long 152°25′ W and lat 56° 25′ N); on bottom in 284 m; 12 June 1969; Ted Shigyo, U.S. fishery observer on Japanese stern trawler *Kirishima Maru*. California Academy of Sciences, San Francisco, Calif. (Number CAS 24857).

[2] Museum designations: AB—National Marine Fisheries Service Biological Laboratory, P.O. Box 155, Auke Bay, Alaska 99821; BC—University of British Columbia Institute of Animal Resource Ecology, Vancouver 8, British Columbia, Canada; CAS—California Academy of Sciences, Golden Gate Park, San Francisco, Calif. 94118; SIO—University of California, San Diego, Scripps Institution of Oceanography, P.O. Box 109, La Jolla, Calif. 92037; USNM—U.S. National Museum, Washington, D.C. 20560.

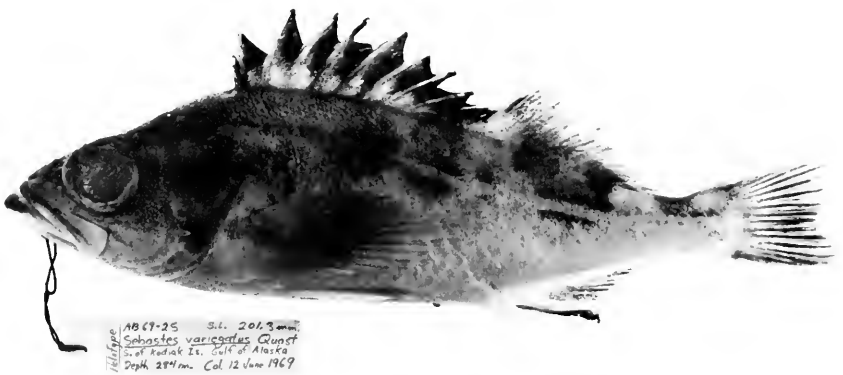

FIGURE 1.—*Sebastes variegatus* sp. n., holotype CAS 24857; 201 mm; male.

FIGURE 2.—*Sebastes variegatus* sp. n., paratypes USNM 204937 (upper) and SIO 70-171 (lower); 185 and 193 mm; both males. (Depth and date of Paratype 2 should be the same as for Paratype 1.)

PARATYPES (NOS. 1 and 2)

(Original number AB 69-26); U.S. National Museum 204937, 185 mm, and SIO 70-171 University of California, San Diego, 193 mm; males; Gulf of Alaska south of Kodiak Island (approximately long 152°25′ W and lat 56°25′ N); on bottom in 240 m; 13 June 1969; Ted Shigyo, U.S. fishery observer on Japanese stern trawler *Kirishima Maru.*

OTHER MATERIAL

WESTERN GULF OF ALASKA. Davidson Bank: AB 68-598 (3, 175-182 mm SL), 26 October 1968, RV *Miller Freeman.* East of Simeonof Is.: BC 65-70 (2, 145-196 mm SL), 5 August 1964, *G. B. Reed.* Kodiak Island vicinity: AB 67-171 (1, 235 mm SL), 19 April 1964, *Taiyo Maru No. 81*; AB 67-22 (1, 277 mm SL), 21 April 1967, *Yutaka Maru;* AB 66-154 (1, 286

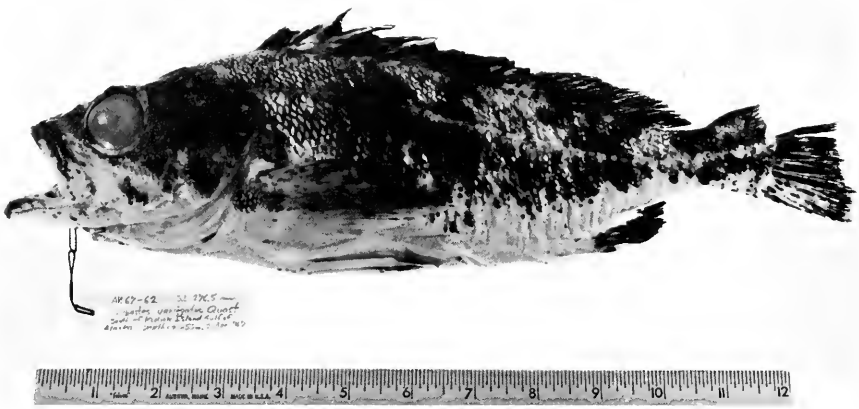

FIGURE 3.—Melanistic specimen of *Sebastes variegatus* (AB 67-62); 277 mm SL; Kodiak Island vicinity.

mm SL), 8 May 1966, *Taiyo Maru No. 2;* AB 67-68 (1, 210 mm SL), 28 April 1967, *Yutaka Maru;* AB 64-847 (1, 153 mm SL), 10 September 1964, *Taiyo Maru No. 77;* AB 68-10 (1, 197 mm SL), 3 September 1964, *Taiyo Maru No. 77;* AB 63-120 (2, 137-143 mm SL), 4 August 1962, RV *Yaquina.* MIDDLE GULF OF ALASKA. Portlock Bank (S of Seward): AB 63-123 (2, 157-230 mm SL), 13 August 1962, RV *Yaquina;* BC 64-292 (1, 223 mm SL), 26 August 1963, *G. B. Reed.* Vicinity of Port Bainbridge (ESE of Seward): AB 64-651 (2, 120-132 mm SL), 16 July 1963, RV *Yaquina.* West of Montague Is.: AB 64-645 (1, 109 mm SL), 16 July 1963, RV *Yaquina.* South of Montague Is.: AB 68-527 (5, 147-169 mm SL), 18 July 1968, RV *Oshoro Maru.* Off Cape Yakataga: AB 68-526 (1, 193 mm SL), 25 July 1968, *Daishin Maru No. 12.* Off Yakutat: AB 70-23 (2, 245-278 mm SL), 15 July 1968, RV *Oshoro Maru.* EASTERN GULF OF ALASKA. Queen Charlotte Sound: BC 70-2 (1, 237 mm SL), 12 June 1970, RV *G. B. Reed* (Electrophaerogram). Frederick Sound: AB 67-11 (3, 241-266 mm SL), 28 February 1967, RV *Murre II.* Forrester Is. vicinity: AB 68-524 (1, 211 mm SL), 24 July 1968, *Ishikari Maru;* AB 68-525 (1, 242 mm SL), 24 July 1968, *Ishikari Maru.* West of Dall Is.: AB 68-24 (3, 146-171 mm SL), 5 November 1956, RV *John N. Cobb.*

DIAGNOSIS

A species of *Sebastes* with body outline slender and symmetrically terete; preocular, postocular, tympannic, and parietal spines present and supraocular, coronal, and (usually) nuchal spines absent; second anal spine longer than third; symphyseal knob not prominent; 18 (rarely 17 or 19) rays in pectoral fin; black spinous dorsal and caudal fin membranes; peritoneum dark brown to jet black; and dark blotched pattern of pigmentation on back and sides, the pattern partly interrupted by a broad unpigmented band that extends over posterior 2 3 of body and includes lateral line.

MORPHOLOGY (PRINCIPALLY FROM HOLOTYPE AND PARATYPES)

Body form, including head, slender, the outline smooth and tapers nearly symmetrically

to body axis anteriorly and posteriorly. Body near head flattened laterally, body width slightly greater than 1/2 body depth at pelvic fins. Interorbital region flat to convex. Cranial ridges over orbit usually lower than dorsal profile of head when viewed from side, but sometimes tangent or project slightly above the profile. Dorsal outline of head usually smooth and slightly concave between snout and nape. Lower jaw projects and enters dorsal profile of head. Symphyseal knob weakly developed or absent. Posterior profile of caudal fin indented; that of anal fin slants upward-posteriorly. Pectoral fin symmetrical; both it and pelvic fin extend to or nearly to vent.

Head spines low but strong and well developed—nasal, preocular, postocular, tympannic, and parietal present. Other cranial spines absent except occasionally one nuchal present. Two opercular spines diverge slightly and are contained within outline of opercular flap. Some specimens with one (rarely two) small spines on lower opercle. Two strong suprascapular spines. Five preopercular spines: upper three longest and diverge slightly; second from top longer than either first or third. Lower two preopercular spines broadly triangular and strong. Lacrimal bone has two blunt or rounded prominences ventrally but no spines. Spines absent on suborbital bones and stay.

Spinous rays in dorsal fin strong and sharp, third to fifth usually longest. Interspinous membranes markedly indented posteriorly in each space; indention varies from about 1/2 of length of following spine in first to about 1/4 of following spine in fourth space and posteriorly. Spinous rays of anal and pelvic fins strong and prominent; second anal spine longest, and 14-23% of its length exceeds third. Each soft ray in dorsal fin branched at least once, usually with posterior and sometimes anterior branchlet divided again. Bordering principal rays on caudal fin simple, but branched rays have three sets of dichotomies each (end in eight branchlets). Soft rays of anal fin branched at least once, and usually each branchlet divided again; sometimes posterior branchlet divided several times. Soft rays of pelvic fin branched at least twice, usually with further dichotomies. Uppermost ray of pectoral fin simple; ventrally, degree of branching increases from one dichotomy to 1-1/2 or 2; lowermost rays slightly thickened and simple, rarely with one dichotomy.

Squamation on head ctenoid and nearly complete—includes snout between premaxillary processes (in holotype but not in paratypes), sides of snout, dorsal surface of head, preopercle, opercle, lacrimal area, maxilla, mandible, and branchiostegals. Lips and branchiostegal membranes not scaled. Body fully scaled; scales finely ctenoid on body sides, but ctenation reduced on belly and breast and even more reduced to absent on scales of isthmus, which are all small. A few small, smooth scales extend in line posteriorly from axilla of each pelvic fin. Scales on bases of vertical fins and between pelvic fins markedly reduced in size. Scaled areas on dorsal fin spines extend nearly to tips. Interspinous membranes usually scaled on proximal 1/3; scaled areas usually extend farther distally near fin spines. Dorsal fin squamation mostly smooth on holotype but mostly ctenoid on paratypes. Scales usually absent in areas of dorsal fin membranes with heavy black pigmentation. Rays of soft dorsal fin scaled nearly to tips, but membranes not as completely scaled. Scaled areas of soft dorsal fin usually limited to proximal 2/3 of fin—all scales ctenoid. Squamation of caudal fin ctenoid, its membranes scaled nearly to end of fin. Posterior unpigmented border of fin naked. Caudal rays scaled only near their bases (but scales possibly lost from more distal portions of rays in specimens of this series owing to collection methods). Squamation of anal fin ctenoid; fin spines scaled nearly to tips; interspinous membranes naked except for narrow area bordering third spine. Membranes between anal soft rays scaled over proximal 1/2-3/4; scaled areas extend farther distally on soft rays. Pelvic fin has spines and soft rays scaled nearly to tips; scales mostly smooth. Upper pectoral rays scaled nearly to tips, but squamation absent at unpigmented tips of rays. Proceeding downward on ventral 1/2 of pectoral fin, rays have progressively more naked area distally; extent of naked area pro-

gresses from about 1/4 length of uppermost single ray to about 1/2 of lowermost ray.

Upper jaw teeth simple and in two modal sizes. A larger outer row, sometimes mainly uniserial, extends nearly length of each premaxillary. Anteriorly, teeth larger yet and band broadens. A medial gap where tooth patches at lower jaw tip meet upper jaw (small papilla in middle of gap). A smaller inner row of teeth, may also be uniserial, in narrow band over most of premaxillary; it loses uniserial character near large anterior teeth, skirts behind them and continues to medial gap. Teeth of lower jaw simple, uniserial, and similar in size to large teeth in row of upper jaw. Anteriorly, lower jaw teeth are larger and in a tuft that is separated from midline by narrow gap—with jaws shut, from 1/2 to all of each tuft is visible. Vomerine teeth small, in broad V-shaped patch, and sometimes slightly enlarged to suggest a tuft at apex of patch. Palatine teeth small, uniserial except anteriorly where row broadens. Gill rakers slender and pointed, one at bend of arch steps 1.6-2.2 (mode 1.8) into orbit. A small slit behind last gill arch.

A large prenarial pore on side of snout in angle formed by premaxillary process and premaxilla. A larger subnarial pore on flat shelf immediately below nares—outer margin of shelf formed by part of dorsal margin of lacrimal bone. Smaller pores occur singly above nares and below nasal spine. Two large pores on ventral surface of lacrimal bone—one slightly anterior to vertical through subnarial pore and another slightly posterior to this vertical and beneath anterior blunt projection on ventral surface of lacrimal bone. Four prominent pores on each mandible—first in or near fold formed by symphyseal knob and remaining three in a line along ventral surface.

Suborbital bones continuous around eye; second (suborbital stay) conforms to Type 1 of Matsubara (1943a:10-13), i.e. stay tapers to point posteriorly and does not reach preopercle. Sensory canal terminates at midlength of stay. Third and fourth suborbitals tubular. Seven branchiostegals, 5^1_2 on ceratohyal and 1^1_2 on epihyal.

COLOR

Background color of body (based on Kodachrome transparencies of seven freshly caught young adult specimens from Davidson Bank, near Unimak Pass, and the Yakutat vicinity) varies from light pink or light purple-pink to deep red, masked with irregular pattern of dark pigmentation that varies between specimens from barely detectable grey through brown to melanistic black. Masking nearly absent on one large individual with red coloration. Some individuals had faded, nearly colorless appearance. Pattern of black pigmentation on sides below dorsal fin basically three large irregular areas extending from midline to or onto fin. One pigmented area beneath middle and another beneath last 1 4 of spinous dorsal fin—first slightly interrupted by narrow light area where it crosses lateral line; second broadly interrupted by light band reminiscent of band along lateral line of *S. proriger*. Third large pigmented area beneath soft dorsal fin forms irregular circle with light spot at center, continues onto lower 1 2 of soft dorsal fin, interrupted near lower border by broad light-colored band along lateral line. Band extends over posterior 2 3 of body. Small pigmented area on dorsal caudal peduncle extends downward to but not across lateral line and forms anterior boundary of pupil-sized lighter spot on dorsal shoulder of caudal fin. Head with diffuse brown to black shading from symphyseal knob and nearby portions of lower jaw to nape; on snout, shaded area extends ventrally to a line through 7 o'clock position on eye. Behind eye, dark shading extends ventrally to about 5 o'clock position and posteriorly over preopercle. Nape dusky to a line between 1 o'clock position of eye and anterior lateral line. Opercle with two broad pigmented bands whose converging axes meet when projected into lower hemisphere of eye. Upper band includes both opercular spines; lower extends toward pectoral base and includes upper two preopercular spines. Lower band aligns generally with irregular pigmented areas on upper 1 2 of pectoral base. On cheek of most specimens a short pigmented streak extends posteriorly and slightly downward from upper

corner of maxilla. Lower cheeks, jaws, and opercular membranes suffused with pink, rose-pink, or red. Spinous dorsal fin with distal 1/2 to 2/3 of interspinous membranes black; remainder above back has pink-related background color of body. Dark pigmentation absent in skin over fin spines, contrasting with dark interspinous membranes. Soft dorsal fin usually distinguished by continuation of body pigmentation onto its lower half where pigmentation forms flattened upper 1/2 of irregular doughnut- or tire-shaped mark. Distally on soft dorsal fin, interspinous membranes darkly pigmented near rays; intervening areas some shade of translucent pink or red. Dark pigmentation terminates equally along fin to form translucent pink border. Caudal fin membranes almost entirely black. Caudal fin terminates with narrow clear or translucent pink or red border. Anal fin pink or red, usually with narrow area or streak of black pigmentation between spines II and III; soft-rayed portion has dusky pigmentation on distal 1/2 of membranes. Background color of pectoral fin same as body but masked by dusky pigmentation in large circular or oval area on upper 2/3 of fin. Small dark pupil-sized spot usually near insertion of rays near midline of fin. Pelvic fin colored as belly.

In preserved specimens dark areas of head, body, and fins persist and include black membranes in spinous dorsal and caudal fins, black stripe between anal spines II and III, dusky pigmentation of lower jaw tip, radiating bands on opercle, black spot in center of pectoral fin, doughnut- or tire-shaped mark below soft dorsal fin, and broad light stripe that accompanies lateral line over posterior 2/3 of body and interrupts two of three large dark areas on body sides. Peritoneum normally dark brown to jet black (one specimen had brown peritoneum with black spots); a melanistic specimen (Figure 3) had light brown peritoneum with black spots).

MERISTIC CHARACTERS

See Table 1.

SIZE OF BODY PARTS

Metrical data on 30 characters are summarized in Tables 2-4. Two methods for fitting regressions of measurements on standard length

TABLE 1.—Counts for meristic characters for holotype of *Sebastes variegatus* and ranges of frequent and infrequent counts for all specimens.

Character	Holotype	Specimens including holotype	
		N	Counts and their frequencies (*N*)
Precaudal vertebrae	10	3	10
Caudal vertebrae (including urostyle)	17	3	17
Dorsal fin spinous rays	13	38	13(36); 14(2)
Dorsal fin soft rays	14	38	13(1); 14-15(37)
Total rays in dorsal fin	27	38	27-28
Anal fin soft rays	7	38	[1]7
Lower single pectoral rays (2 sides)	8, 9	75	7(3); 8-9(72)
Total pectoral rays (2 sides)	18, 18	75	17(4); 18(70), 19(1)
Pored lateral line scales on body (2 sides)	49, 51	73	42(1); 43-51(71), 52(1)
Pored lateral line scales on caudal fin (2 sides)	1, 1	73	0(1); 1(70); 2(2)
Scale rows below lateral line (2 sides)	58, 58	45	46(1); 47-57(42); 58(2)
Rakers on upper limb	11	38	9(1); 10-12(37)
Rakers on lower limb, including raker at bend	28	38	26-28(37); 29(1)
Total rakers	39	38	36-40(37); 41(1)

[1] One of two additional specimens, examined subsequently, had 6 soft rays in the anal fin.

TABLE 2.—Measurements (mm) of type material, *Sebastes variegatus*.

Character	Holotype (SL = 201)	Paratypes	
		No. 1 (SL = 185)	No. 2 (SL = 193)
Head length	70.0	63.0	64.6
Upper jaw length	32.3	28.8	29.3
Prenarial pore length	1.7	1.0	1.4
Subnarial pore length	2.4	2.5	2.5
Orbit diameter	22.4	18.5	19.0
Interorbital width	14.2	13.4	13.7
Raker length	12.0	10.7	12.0
Lower jaw projection	3.8	3.3	3.6
Snout length	17.5	16.4	17.0
Suborbital width	2.5	2.7	2.2
Head width	30.2	29.5	29.8
Pectoral fin length	60.0	53.1	56.7
Pectoral base length	19.0	18.3	19.2
Caudal peduncle depth	18.0	15.9	16.0
Dorsal caudal peduncle length	28.7	26.0	26.5
Ventral caudal peduncle length	44.3	40.2	40.9
Body depth (pelvic)	58.4	56.5	56.5
Body depth (anal)	47.5	45.2	48.9
Anal spine I length	18.5	16.9	16.5
Anal spine II length	40.2	36.6	37.8
Anal spine III length	33.4	30.9	30.8
Soft dorsal fin base length	52.3	43.7	45.3
Anal fin base length	32.1	27.6	31.0
Pelvic fin spine length	31.7	28.8	30.3
Pelvic fin length	48.0	42.7	44.5
Pelvic insertion to midvent	45.4	46.5	45.6
Midvent to anal fin	11.5	11.0	11.3
Longest dorsal fin spine length	29.8	29.3	28.2
Longest dorsal soft ray length	29.2	26.7	27.2
Longest anal soft ray length	37.9	35.7	35.4

TABLE 3.—Regression of measurements (Y) on SL (X) in the point-slope form of the log-transformed allometric equation $Y = aX^b$ over the interval 109<SLmm<286, *Sebastes variegatus*.

Character (No. specimens)	$\bar{X}$	$\bar{Y}$	$b(\pm 95\%)$[1]	S_{yx}	r^2
Head length (38)	2.26567	1.80164	1.03704(±0.02555)*	0.0100	0.995
Upper jaw (38)	2.26567	1.46445	1.00526(±0.03700)n.s.	0.0100	0.988
Prenarial pore (38)	2.26567	0.12076	0.68961(±0.22125)*	0.4499	0.526
Subnarial pore (38)	2.26567	0.36358	0.64076(±0.15785)*	0.0510	0.653
Orbit (38)	2.26567	1.29809	0.89650(±0.07000)*	0.0224	0.949
Interarbital (38)	2.26567	1.10458	1.17465(±0.05915)*	0.0200	0.978
Raker (37)	2.26051	1.02983	0.99778(±0.09776)n.s.	0.0300	0.926
Lower jaw projection (35)	2.26552	0.59671	1.40000(±0.18610)*	0.0583	0.876
Snout (35)	2.26552	1.20640	1.05453(±0.05560)n.s.	0.0173	0.978
Suborbital (35)	2.26552	0.39634	0.76861(±0.17185)*	0.0539	0.714
Head width (34)	2.27223	1.47624	1.03266(±0.07375)n.s.	0.0224	0.962
Pectoral fin (35)	2.26539	1.71905	0.96682(±0.04611)n.s.	0.0115	0.982
Pectoral base (38)	2.26539	1.24749	0.95907(±0.04845)n.s.	0.0144	0.979
Caudal peduncle depth (35)	2.25292	1.21462	0.93501(±0.05720)*	0.0173	0.971
Dorsal caudal peduncle (35)	2.26552	1.42222	0.98447(±0.06855)n.s.	0.0224	0.962
Ventral caudal peduncle (35)	2.26552	1.59340	0.92085(±0.04905)*	0.0141	0.978
Body depth (pelvic) (38)	2.26567	1.74993	1.01305(±0.06455)n.s.	0.0200	0.966
Body depth (anal) (35)	2.26552	1.66765	0.89023(±0.06100)*	0.0200	0.964
Anal spine I (38)	2.26567	1.20583	0.81995(±0.10465)*	0.0332	0.876
Anal spine II (37)	2.26051	1.54643	0.73519(±0.06340)*	0.0200	0.940
Anal spine III (35)	2.26552	1.46436	0.83416(±0.05255)*	0.0173	0.968
Soft dorsal base (35)	2.26552	1.63887	0.90102(±0.10955)n.s.	0.0346	0.894
Anal base (35)	2.26552	1.46978	0.81406(±0.07225)*	0.0224	0.941
Pelvic fin spine (34)	2.26648	1.45182	0.85218(±0.04725)*	0.0141	0.976
Pelvic fin (38)	2.26567	1.62047	0.93185(±0.03595)*	0.0100	0.987
Pelvic insertion to midvent (38)	2.26567	1.63481	1.15544(±0.11405)*	0.0361	0.922
Midvent to anal fin (35)	2.26552	1.00595	1.10201(±0.25370)n.s.	0.0794	0.703
Longest dorsal fin spine (36)	2.26180	1.40658	0.94004(±0.09285)n.s.	0.0283	0.926
Longest dorsal soft ray (34)	2.26648	1.42402	0.96599(±0.08310)n.s.	0.0265	0.945
Longest anal soft ray (35)	2.26552	1.54610	0.97441(±0.04500)n.s.	0.0141	0.983

[1] Asterisk indicates a difference in the slope exponent (b) significantly different from unity at the 95% level; n.s. indicates no significant difference at this confidence level.

were explored, the power or allometric equation, $Y = aX^b$, in $\log_{10}$ transformed form, and the first degree or rectilinear equation, $Y = a + bX$, untransformed. Both functions were fit by a computer regression program (Sokal and Rohlf, 1969:696). Degree of fit was judged by the "coefficient of percentage variation explained by regression," the square of the correlation coefficient for regression, r^2 (Table 3). In 18 of the 30 characters, nearly identical r^2's were obtained by the alternative equations, including 9 characters for which the allometric exponent differed in a minor (<0.20) but significant degree from unity at the 95% confidence level, i.e. the growth relationships seemed to depart significantly from isometry. Plots of selected examples from the nine characters showed only minor differences between the two functions in these instances. In four comparisons, three of which showed significant departure from unity in the allometric exponent (subnarial pore, lower jaw projection, and suborbital), the first-degree equation appeared to give a superior fit. For the remaining eight comparisons, the allometric equation was superior.

In the light of these considerations, the $\log_{10}$ transformations of the allometric equation ($\log Y = \log a + b \log X$) was chosen for general application because it is more versatile and gave a satisfactory fit in all comparisons. Those few characters which were fit somewhat better by the first-degree equation appear to be of relatively minor utility in rockfish systematics. Parameters are presented in the point-slope form of the transformed allometric equation ($Y = \bar{Y} + b\ (X - \bar{X})$, where $Y = \log_{10} Y$ and $X = \log_{10} X$) to emphasize the working interval about the means of the variates and to de-emphasize the Y-intercept which has no theoretical value in these representations.

The measurements are also presented in the form of 95% confidence limits for proportions of future individual specimens, based on the material examined (Table 4). The limits were

TABLE 4.—Upper and lower 95% confidence limits for single future observations on proportions (percent of SL), based on the material examined.[1]

Character	Standard length (mm)				
	120	160	200	240	280
Head length	35.52 32.19	35.84 32.59	36.13 32.86	36.40 33.06	36.64 33.22
Upper jaw	16.56 15.01	16.56 15.06	16.58 15.08	16.61 15.08	16.64 15.08
Prenarial pore	1.16 0.58	1.05 0.53	0.98 0.50	0.93 0.47	0.89 0.44
Subnarial pore	1.88 1.14	1.68 1.04	1.55 0.96	1.46 0.89	1.39 0.84
Orbit	12.57 10.09	12.16 9.83	11.88 9.61	11.67 9.42	11.52 9.25
Interarbital	7.06 5.80	7.40 6.12	7.70 6.37	7.96 6.57	8.19 6.73
Raker	6.82 5.08	6.78 5.10	6.78 5.10	6.79 5.08	6.81 5.07
Lower jaw projection	2.41 1.35	2.68 1.53	2.92 1.68	3.16 1.80	3.38 1.90
Snout	9.29 7.83	9.41 7.97	9.52 8.07	9.63 8.14	9.73 8.20
Suborbital	1.95 1.14	1.81 1.08	1.71 1.03	1.65 0.98	1.60 0.94
Head width	17.63 14.10	17.71 14.30	17.83 14.41	17.97 14.48	18.11 14.51
Pectoral fin length	30.52 27.23	30.17 27.03	29.95 26.83	29.79 26.65	29.67 26.48
Pectoral base	10.46 9.11	10.32 9.02	10.22 8.94	10.15 8.86	10.11 8.80
Caudal peduncle depth	10.24 8.63	10.02 8.49	9.87 8.37	9.77 8.26	9.70 8.16
Dorsal caudal peduncle	16.13 12.93	16.00 12.92	15.94 12.88	15.91 12.83	15.91 12.76
Ventral caudal peduncle	23.60 20.52	23.02 20.11	22.61 19.76	22.31 19.46	22.07 19.20
Body depth (pelvic)	33.46 27.49	33.48 27.68	33.56 27.77	33.69 27.80	33.83 27.80
Body depth (anal)	29.21 23.97	28.20 23.30	27.51 22.75	27.01 22.27	26.61 21.84
Anal spine I	11.08 8.00	10.46 7.64	10.05 7.34	9.74 7.09	9.51 6.87
Anal spine II	23.81 19.55	21.99 18.18	20.72 17.14	19.77 16.31	19.03 15.62
Anal spine III	18.49 15.58	17.58 14.90	16.93 14.36	16.45 13.92	16.06 13.54
Soft dorsal fin base	29.25 20.77	28.26 20.31	27.63 19.87	27.20 19.47	26.89 19.11
Anal fin base	21.70 13.85	20.39 13.24	19.43 12.79	18.68 12.43	18.07 12.14
Pelvic fin spine	17.52 15.23	16.75 14.63	16.20 14.16	15.78 13.77	15.45 13.44
Pelvic fin length	24.68 22.38	24.17 21.98	23.80 21.65	23.52 21.37	23.30 21.12
Pelvic insertion to midvent	26.13 18.33	27.16 19.28	28.11 19.97	28.98 20.50	29.80 20.92
Midvent to anal fin	7.80 3.56	7.92 3.71	8.09 3.80	8.29 3.85	8.49 3.88
Longest dorsal fin spine	16.46 12.45	16.10 12.30	15.88 12.14	15.74 11.98	15.64 11.83
Longest dorsal soft ray	16.63 12.79	16.39 12.73	16.26 12.64	16.19 12.54	16.15 12.44
Longest anal soft ray	20.69 17.99	20.49 17.90	20.37 17.80	20.29 17.70	20.24 17.61

[1] Based on confidence belts for individual predicted measurements (Sokal and Rohlf, 1969:422) from the parameters for regression (Table 3). Data were back-transformed to arithmetic values for computation of proportions. If limits are desired in the form of measurements, the percentages may be multiplied by the appropriate SL.

obtained from 95% confidence limits for fits to regression (Table 3) back-transformed to the original variates and converted to percent of standard length. Marr (1955) discussed the disadvantages of using proportions in systematics, particularly when size-specific changes are not identified, but neglected several advantages. Size-specific changes are here identified, and the presentation of data in the form of proportions facilitates rapid comparisons between specimens and allows data on measurements to be presented more economically than in graphs. Proportions vary much less with length than original measurements, sometimes surprisingly little when the numerous factors that influence their variation are considered (Table 4), and therefore allow easier interpolation between tabulated reference points than representations of original variates. Also, the continued use of proportions in fish systematics, often without indication of size-specific changes, attests to a prevailing opinion that proportions are useful despite their occasional misuse and other drawbacks.

Plots of the limits for proportions (Table 4) disclose that they are slightly curvilinear and asymmetrical from left to right. These characteristics reflect the y-intercept effect discussed by Marr (1955) combined with the effects of normal divergence of confidence belts in regression with distance from the combined mean, allometry between the original variates, and distortion caused by back-transformation from the logarithm of a function. Size-specific confidence limits for proportions whose allometric coefficients did not differ significantly from unity (Table 3) are included in Table 4 because the calculated value is a better estimate of the exponent than is arbitrarily assumed isometry.

AXIAL SKELETON

X-rays of types show 27 vertebrae, including urostyle (Table 1). Pterygiophores of spinous dorsal fin single in spaces between neural spines except between neural spines 2 and 3, which contains pterygiophores of dorsal fin spines 2 and 3. Pterygiophores of soft dorsal fin usually doubled in interneural spaces except single pre-

ceding neural spines 5 and 8 or 9. Caudal skeleton apparently with hypurals 2 and 3 and 4 and 5 fused into upper and lower plates, respectively, as determined for other scorpaenid representatives by Quast (1965:580). Point of enlarged pterygiophore that supports anal fin spines I and II contacts haemal arch of 11th vertebra, here considered the first caudal vertebra.

ELECTROPHORETIC PATTERN

A standardized starch gel electrophaerogram of haemoglobin from a male adult *S. variegatus* (BC 70-2, from Queen Charlotte Sound, British Columbia), with comparative material on *S. zacentrus*, was kindly furnished by Henry Tsuyuki of the Fisheries Research Board of Canada (Figure 4).[3]

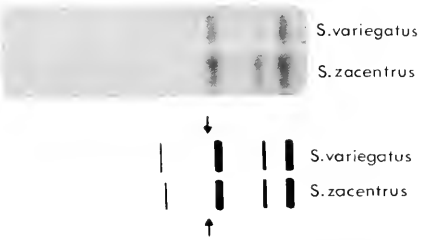

FIGURE 4.—Comparative starch gel electrophaerograms of the haemoglobins from *S. variegatus* and *S. zacentrus* furnished by Henry Tsuyuki, Fisheries Research Board of Canada (see text). The arrow represents the origin in the schematized pattern. The anode is to the right and the cathode to the left. The minor cathodal zone, which Tsuyuki found to be consistently diagnostic for the two species, could not be reproduced photographically and is shown in schematic form in approximately the concentrations found.

[3] Vancouver Laboratory, 6640 NW. Marine Drive, Vancouver 8, British Columbia, 28 July 1970. "Erythrocytes were washed once with a 1% sodium chloride solution before hemolysis to avoid contamination from serum proteins. Haemoglobins from washed and unwashed red cells possessed identical patterns." For methods used in obtaining blood samples and for electrophoresis, see Tsuyuki et al. (1968). Identity of the *S. variegatus* specimen verified by the author; however, the specimen had a head size much larger than normal (38.3% of SL) and was the only specimen out of 40 seen that had 6 soft rays in the anal fin instead of 7.

SIMILAR SPECIES

Sebastes variegatus resembles *S. emphaeus*, *S. proriger*, *S. saxicola*, *S. wilsoni*, and *S. zacentrus* in morphology, morphometry, meristics, and coloration. The six species appear to be closely related members of a complex that generally shares the characters of *S. variegatus* other than the pectoral count of 18, the unpigmented band that encloses the lateral line and extends about 2/3 along the body sides, and the black coloration of the spinous dorsal and caudal fin membranes. The complex may also include *S. dalli*, *S. elongatus*, *S. jordani*, and *S. semicinctus* which also seem to resemble the six species, but these appear to be easier to differentiate from *S. variegatus* and I do not further consider them here. Although the analyses of rockfish haemoglobin and muscle proteins by Tsuyuki et al. (1968) shed little light on the group's validity, subsequent work by Tsuyuki (see footnote 3) suggests a close relationship between *S. variegatus* and *S. zacentrus* (Figure 4). The possibility that *S. variegatus* represents a hybrid may be dismissed because of a normal degree of variation in measurements, distinctiveness and consistency of pectoral ray counts and body coloration, abundance of specimens, and normal reproduction as indicated by normally developed gonads and gravid females.

As a natural group, the six species do not appear to coincide with those at the subgeneric level erected by Cramer, Eigenmann, and Beeson, or Jordan and Evermann, as described by Jordan and Evermann (1898, II: 1765-1777). However, in some respects it does resemble the subgenus *Hatumens*, erected by Matsubara (1943b: 192) under *Sebastes*, and the type species, *Sebastodes owstoni*, as figured by Jordan and Thompson (1914:pl. 31, fig. 3).

Ranges of meristic characters for most species in the hypothetical group (from Phillips (1957) plus personal examination of representatives—Table 5) either are nearly identical (soft rays in the dorsal, anal, and pectoral fins) or overlap broadly (pored scales in the lateral line, gill rakers, and scale rows below lateral line). In characteristics for which most of the group are nearly identical, *S. variegatus* deviates mark-

TABLE 5.—Data and material for species comparisons with *Sebastes variegatus*.

Species	Phillips (1957)			Material examined		
	No. specimens	Meristics		No. specimens	Meristics	
		No.	SL		No.	SL
S. emphaeus	--	--	--	7	7	101-131
S. proriger	7	6	230-389	12	12	161-270
S. saxicola	35	9	103-308	--	--	--
S. wilsoni	3	3	102-133	6	6	50-174
S. zacentrus	15	10	166-338	20	20	133-281

edly only in pectoral count. In those species where means of characteristics differ noticeably but ranges overlap broadly, *S. variegatus* usually is of central value, which suggests that it may be a more generalized member of the group.

Comparisons of morphometric characters between *S. variegatus* and the other species lead to a similar conclusion. Ranges for the species overlap broadly, based on Phillips (1957) and my own measurements (head length, longest dorsal fin spine, least depth of caudal peduncle, pelvic insertion to vent, raker at bend of gill arch, width of pectoral fin base, body depth at pelvic girdle, bony interorbital, orbit, length of pectoral fin, length of pelvic fin, and length of upper jaw—in percent of standard length).

To assist differentiating *S. variegatus* from similar species a table of discriminatory characters is presented (Table 6).

GEOGRAPHIC AND BATHYMETRIC RANGE

Present known range of *S. variegatus* is from Unimak Pass, Aleutian Islands, to Goose Island Bank, Queen Charlotte Sound[4] (Figure 5). Depth of capture ranges from 70 to 305 m.

ACKNOWLEDGMENTS

William I. Follett (California Academy of Sciences), Warren C. Freihofer (Stanford University), and Norman J. Wilimovsky (University of British Columbia) gave permission for examination of collections or shipped material.

[4] The record for Queen Charlotte Sound was obtained by Sigurd J. Westrheim, Fisheries Research Board of Canada, Nanaimo Research Station, Nanaimo, British Columbia, on the RV *G. B. Reed* during Cruise GBR 70-1 (personal communication, 5 August 1970).

TABLE 6.—Summary of salient comparative features of *Sebastes variegatus* with five similar species. (Proportions of *S. variegatus* refer to 95% confidence limits of Table 4. Source and quantity of data on other species are summarized in Table 5. Code for sources: *, Phillips (1957); **, original material (Tables 1-4); ***, both sources. In some instances my data on northeastern Pacific specimens differ from Phillips (1957) in degree of discrimination.)

S. emphaeus.
Counts: Pectoral rays 17 (occas. 18)**, *variegatus* 18 (occas. 17); scale rows ≤46*, *variegatus* ≥46. Percent of SL (apply to interval of size overlap, 109-131 mm): Upper jaw ≤15.0**, *variegatus* ≥15.0; ventral caudal peduncle ≤20.8**, *variegatus* ≥20.5; anal spine I ≤8.3**, *variegatus* ≥8.0; anal spine II ≤19.4**, *variegatus* ≥19.6; anal spine III ≤15.8**, *variegatus* ≥15.6. Pigmentation: Blotches on sides not interrupted by an unpigmented band along lateral line; no black area on fin membrane between anal spines II and III (both characteristics from pl. 31 of Starks, 1911).

S. proriger.
Counts: Pectoral rays 17*** (occas. 18)**, *variegatus* 18 (occas. 17); scale rows ≥55* (47-56)**, *variegatus* ≤58. Percent of SL (apply to interval of size overlap 161-270 mm, for Quast data): Upper jaw ≤14.7* (≤15.7)**, *variegatus* ≥15.1; orbit ≤9.3* (≤10.5)**, *variegatus* ≥9.3; anal spine II ≤15.4* (≤16.4 but normally below 95% limits of *variegatus* at size [Table 4])**; anal spine III (below 95% limits of *variegatus* at size [Table 4])**; pelvic fin (below 95% limits of *variegatus* at size [Table 4])**; longest dorsal soft ray ≤12.5*, *variegatus* ≥12.4; longest anal soft ray ≤16.1*, *variegatus* ≥17.6. Pigmentation: Unpigmented bond along lateral line extends to head.

S. saxicola.
Counts: Dorsal soft rays 12 (occas. 13)*, *variegatus* 14 or 15 (occas. 13); pectoral rays 16 (occas. 15 or 17)*, *variegatus* 18 (occas. 17); lateral line pored scales ≤42*, *variegatus* ≥42, rakers ≤34*, *variegatus* ≥36. Percent of SL: head ≥35.7*, *variegatus* ≤36.6; orbit ≥11.8*, *variegatus* ≤12.5; longest anal soft ray ≤18.2*, *variegatus* ≥17.6. Pigmentation: Blotches on sides not interrupted by unpigmented bond along lateral line.

S. wilsoni.
Counts. Anal soft rays 6***, *variegatus* 7 (rarely 6); pectoral rays 16-17* (occas. 18)**, *variegatus* 18 (occas. 17); lateral line pored scales ≤41* (≤43)**, *variegatus* ≥42; scale rows 45-50* (41-45)**, *variegatus* ≥46. Percent of SL (apply to interval of size overlap, 109-174 mm, in Quast data): pelvic fin length ≤22.0**, *variegatus* ≥22.0. Pigmentation: Dark blotches on sides not interrupted by an unpigmented bond along lateral line.

S. zacentrus.
Counts: Pectoral rays 17 (occas. 18)*, (occas. 19)**, *variegatus* 18 (rarely 17 or 19)**; scale rows 43-50* (≤47)**, *variegatus* ≥46; rakers ≤37* (≤38)**, *variegatus* ≥36. Percent of SL (apply to interval of size overlap, 133-281, for Quast data): Upper jaw 15.6-16.9* (normally above 95% limits of *variegatus* at size [Table 41])**; longest anal soft ray ≤17.9*, *variegatus* ≥17.6. Pigmentation: Blotchts on sides not interrupted by unpigmented bond along lateral line.

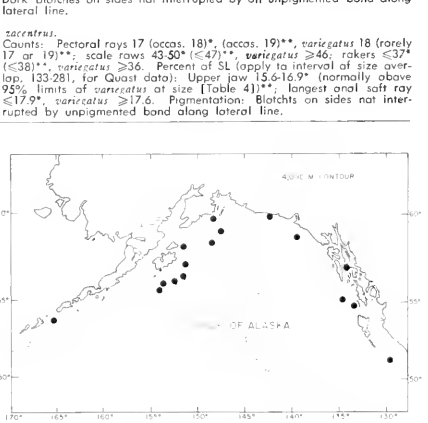

FIGURE 5.—Localities at which *Sebastes variegatus* were captured.

Sigurd J. Westrheim (Fisheries Research Board of Canada, Nanaimo Research Station) furnished fresh material and reviewed the manuscript, and Henry Tsuyuki (Fisheries Research Board of Canada, Vancouver Laboratory) contributed the haemoglobin electrophaerograms. Daniel M. Cohen (United States National Museum), William N. Eschmeyer (California Academy of Sciences), and Bruce L. Wing (National Marine Fisheries Service Biological Laboratory, Auke Bay, Alaska) provided editorial and scientific comments.

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CALICO SCALLOP DISTRIBUTION, ABUNDANCE, AND YIELD OFF EASTERN FLORIDA, 1967-68[1]

RICHARD B. ROE,[2] ROBERT CUMMINS, JR.,[3] AND HARVEY R. BULLIS, JR.[4]

ABSTRACT

During 18 months, from August 1967 to December 1968, the National Marine Fisheries Service Exploratory Fishing and Gear Research Base in Pascagoula, Miss., conducted a comprehensive survey of the calico scallop (*Argopecten gibbus*) grounds off eastern Florida. The survey disclosed various aspects of the life history, distribution, abundance, and yield and annual variation in geographical and depth distribution. Predictions for a fall fishery are possible since distribution and abundance are established at spat set and can be delineated by midsummer. A fall fishery is recommended as catch rates and yield were highest between September and December and decreased rapidly during late winter and spring.

There are two or more species of *Argopecten* called "calico" scallops (Waller, 1969); however, the more common is *A. gibbus* (L.). This is the species involved in our study, occurring from Delaware Bay to the Caribbean Sea and throughout the Gulf of Mexico (Johnson, 1934; Bullis and Ingle, 1959; Carpenter, 1967; Waller, 1969). Calico scallops derive their name from the blotched coloration of their shells which vary from red to light brown on a white background, giving a "calico" effect to the shell.

Three commercial grounds have been delineated by the Exploratory Fishing and Gear Research Base at Pascagoula, Miss., and its Field Station in Brunswick, Ga. These are located off North Carolina (Cummins, Rivers, and Struhsaker, 1962), off eastern Florida near Cape Kennedy (Bullis and Cummins, 1961; Cummins, et al., 1969;[6] Drummond, 1969), and in the northeastern Gulf of Mexico (Bullis and Ingle, 1959; Carpenter, 1967). Explorations had shown a large resource exists, particularly off Florida, but monthly and yearly changes in distribution and abundance had seriously hampered delineation of the resource.

In August 1967 an 18-month survey was initiated on the Cape Kennedy grounds to assess the scallop stock and determine the causes of variation in distribution, abundance, and yield. These grounds were selected because of size, location, and a developing industry.

The survey provided information on the dynamics of the scallop population present at that time, but there is a need for additional studies on life history, age, and growth.

METHODS

Cruises were conducted monthly between August and December 1967, and bimonthly between February and December 1968. Four standard transects, each extending from 10 to 40 fm, were made on each cruise. These were: transect A at lat 28°03′ N, transect B at lat 28°27′ N, transect C at lat 29°03′ N, and transect D at lat 29°25′ N (Figure 1). Tows were also made between transects. An 8-ft scallop dredge, fitted with 2-inch bag rings and 2½-inch mesh nylon liners, was used in the survey. All tows were 30 min.

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[3] National Marine Fisheries Service Exploratory Fishing and Gear Research Station, Brunswick, Ga. 31520.

[4] National Marine Fisheries Service, Washington, D.C. 20240.

[6] Cummins, R., Jr., R. Maurer, L. May, and J. Rivers. 1969. Summary log of scallop locations with predicted catch rates of Cape Kennedy grounds — fall 1969. Issued by Bureau of Commercial Fisheries Exploratory Fishing and Gear Research Field Station, Brunswick, Ga., for limited distribution.

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FISHERY BULLETIN: VOL. 69, NO. 2, 1971.

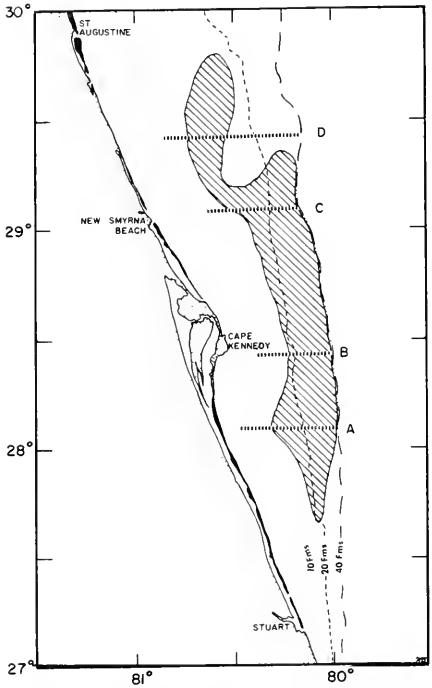

FIGURE 1.—General distribution of calico scallops and four standard transects occupied during the 1967-68 survey off the Florida east coast.

A total of 1,483 drags was made during the survey—285 in transect A, 403 in transect B, 493 in transect C, and 302 in transect D. The following were determined for each drag: total catch in bushels (using a standard steel shrimp basket averaging 70 lb.), number of bushels of live scallops, pounds of meat per bushel, number of pints of meat per bushel, number of meats per pint, and size frequency (measured as shell diameter in millimeters). Randomly selected individuals were examined for gonad coloration and sexual maturation. The data were analyzed with the UNIVAC 9200 computer at the Exploratory Fishing and Gear Research Base at Pascagoula, Miss.

The survey results are subdivided according to spawning, age and growth, mortality, distribution and abundance, and yield.

SPAWNING

Bourne and Bligh (1965) found sea scallop (*Placopecten magellanicus*) ovaries change color during maturation, progressing from pink at early stages to deep coral-red when ripe. Color changes were also noted in calico scallop ovaries, undeveloped ovaries being whitish, ripe ovaries bright reddish-orange.

Yellowish-orange coloration was seen in very few scallops during the August cruise, but by September the rate and incidence of color change was highly noticeable. Coloration and incidence increased into fall and winter and by February ovaries were predominantly reddish-orange or ripe. The majority of ovaries taken in April were bright reddish-orange. June ovaries were largely uncolored.

Sexual maturation in sea scallops begins in March, spawning occurring 7 months or so later in September or October. Calico scallops apparently have a similar maturation period based on color change progression which begins in August and ends in March or April. Protracted spawning does take place in some areas since small numbers of 5 to 30 mm scallops occur throughout the year (Figure 2).

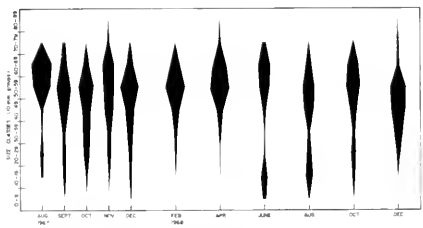

FIGURE 2.—Monthly size/frequency distribution of scallops for the combined transects off the Florida east coast during the 1967-68 survey.

AGE AND GROWTH

Monthly size frequencies are shown in Figure 2. From June until September the distribution is bimodal, but in October the lower mode disappears and a single mode exists until April. This is clearly illustrated in the 1968 data beginning in June when modes occur at 11.9 and 62.1 mm. In August modes are present at 26.0 and 60.6 mm and in October at 34.0 and 61.5 mm. Only one mode at 51.0 mm occurs in December.

The lower mode value gradually increases during this period while the upper mode decreases. These changes reflect a melding of year classes caused by a more rapid growth rate in the younger year class than in the older one. In December the two year classes are indistinguishable. Since year classes cannot be separated in the winter data, it is difficult to determine when the older class disappears. Presumably this occurs some time between December and April.

Growth rates could not be accurately determined from the data because of gear selectivity for the smaller sizes and because year classes were often inseparable in the size-frequency distribution. A growth estimate was derived for a selected bed located on transect C in 22 to 27 fm. The size-frequency data indicate two year classes were present most of the survey period (Table 1). These are more distinct in the separated 1968 data (Table 2).

The mean size increases linearly by about 1.0 to 2.0 mm per month during August to February (Table 1). From April to December the mean gradually decreases because of recruitment from the spring spawn.

TABLE 2.—Monthly size frequency data for transect C, 22 to 27 fm, divided by year class. Classes are 10 mm.

Size class	Year class A				Year class B			
	June	Aug.	Oct	Dec.	June	Aug.	Oct.	Dec.
0-9	6	1	--	--	--	--	--	--
10-19	24	57	2	--	--	--	--	--
20-29	4	163	61	125	--	--	--	--
30-39	--	41	3	260	--	--	--	--
40-49	--	--	--	116	1	--	--	--
50-59	--	--	--	103	44	41	8	--
60-69	--	--	--	--	72	211	103	138
70-79	--	--	--	--	31	38	21	7
Mean	13.9	23.8	25.7	37.8	63.5	64.4	65.5	65.0

Since the age groups interact the means given in Table 1 actually represent a combined year class average. Therefore, the June to December 1968 data have been separated into two year classes based on size (Table 2). Year class A was spawned in the spring of 1968 and year class B, representing the larger sizes, was spawned in the spring of 1967. The monthly mean for year class A increased curvilinearly 24 mm from June to December. Year class B mean increased only 2.0 mm from June to October then decreased slightly in December. The increase appeared linear.

Both year classes show evidence of a sigmoid growth curve. Gibson (1956) gives a sigmoid curve for *Pecten maximus* where time is measured in years rather than months as in calico scallops.

MORTALITY

Calico scallops on the Cape Kennedy grounds experienced light commercial exploitation prior

TABLE 1.—Monthly size frequencies for transect C, 22 to 27 fm, by 10-mm size classes, 1967-68.

Size class	1967					1968					
	Aug	Sept.	Oct	Nov.	Dec.	Feb.	Apr.	June	Aug	Oct	Dec.
0-9	--	--	--	--	--	--	--	6	1	--	--
10-19	1	4	--	--	--	--	--	24	57	2	--
20-29	1	4	1	1	--	--	--	4	163	61	125
30-39	1	3	--	3	--	3	--	--	41	3	260
40-49	2	16	3	7	--	4	38	1	--	--	116
50-59	11	58	72	23	38	295	185	44	41	8	103
60-69	9	42	43	39	25	567	343	72	211	103	138
70-79	2	18	2	7	1	28	46	31	38	21	7
80-89	--	--	--	--	--	--	1	--	--	--	--
Mean	55.2	56.4	57.9	59.1	58.7	61.3	61.0	54.2	45.1	51.9	43.0

to 1967. Landings in 1967 totaled approximately 5,000 bu and although several vessels fished the area in 1968, the overall catch was light. Mortality during the survey period was therefore assumed largely due to natural causes.

Catch curves using Ricker's (1958) method of plotting $\log_e$ (average number per drag per month) were constructed (Figure 3). A linear

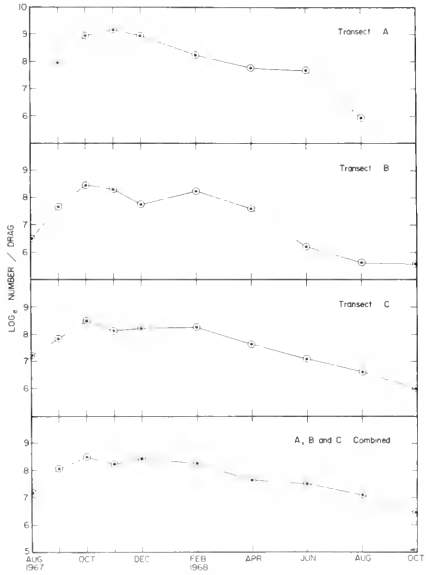

FIGURE 3.—Catch curves for transects A, B, C, and combined.

regression was applied to the descending curve according to Beverton and Holt (1957), using b as an estimate of $F + X$. Since F was negligible, b is an estimate of X or natural mortality. The instantaneous mortality rate (i) is equal to b and the monthly mortality rate (a) was derived from Ricker's appendix.

Data used in the analysis represented the 1967 year class except in transect D where catch data were too sparse for analysis. December 1968 data were not available except for transect C. October 1968 was omitted from transect A because of insufficient data.

The ascending left limb of the curve reflects recruitment. Mortality during this period is difficult to determine because small scallops are inaccessible to the dredge. The dome varied among transects but occurred from October to December or February. The descending limb extended from December to October (August in transect A). All limbs are curvilinear but vary in shape. The curve for transect A is variable. That for transect B is concave from February to June then becomes convex. Transect C curve is almost linear.

Monthly mortality rates, computed assuming a linear relationship (as in Beverton and Holt, 1957), are given in Table 3. Two time periods were treated: one from October to December (the optimum fishing season) and the other from December to October (the descending limb). The latter period includes the spawning season. Although accurate rates for the first period (the dome) could not be accurately ascertained, a crude mortality estimate was desired for the fishing season.

TABLE 3.—Monthly mortality rates (a) and instantaneous mortality rates (i) for transects A, B, and C, individually and combined, for various time periods.

Transect	Time period	i	a
			%
A	10/67-12/67	−0.012	1
	12/67- 8/68	−0.327	27
B	10/67-12/67	−0.365	31
	12/67-10/68	−0.286	25
C	10/67-12/67	−0.150	14
	12/67-10/68	−0.236	21
COMBINED	10/67-12/67	−0.020	2
	12/67-10/68	−0.189	18
C (22-27 fm)	2/68-10/68	−0.231	21

Monthly mortality rates ranged from 1% to 31% for the dome and 18% to 27% for the descending limb. Averages were 12% and 23% respectively.

Age classes were difficult to distinguish and all data may not have been from the 1967 year class. This could have caused differences in the

catch curves. To test this possibility, data from a bed of known age composition were used to construct a catch curve. This bed, located at lat 29°16′ N in 22 to 27 fm on transect C, was established in August 1967 and can be accurately traced through October 1968 with length-frequency data.

The catch curve for the bed (August 1967 to October 1968) is given in Figure 4. Recruitment in late summer and fall was strong. Recruitment and mortality seem balanced from October to February, but neither can be accurately determined. The convex descending limb (February to October) indicates a nonuniform mortality rate, increasing rapidly after June with the termination of spawning.

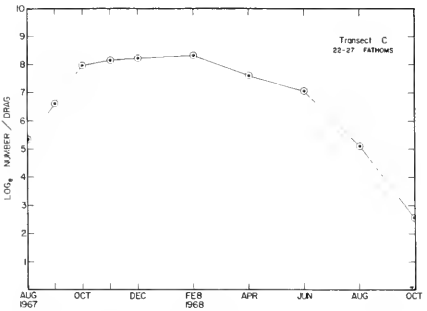

FIGURE 4.—Catch curve for transect C, 22 to 27 fm. Data are for the 1967 year class.

The February to October segment was treated linearly to estimate monthly mortality. Results were $i = -0.231$ and $a = 21\%$ (see Table 1). Mortality rates for the dome (October to February) were not calculated.

Although these rates are similar to those in Table 3, enough discrepancy exists to indicate more than one year class might have been included in Table 3 data, causing some differences in mortality rates. This does not preclude mortality rate differences on the grounds.

The major calico scallop predator appears to be the starfish (*Asterias*) which is often taken in large numbers in dredge tows (Figure 5) and has been seen by submarine observers feeding on scallops (Figure 6). Rays and skates may feed on calico scallops (Struhsaker, 1969) and puffers (*Sphoeroides*) have been taken with numerous small (2 to 5 mm) scallops in their stomachs. Other predators are not known.

Little is known about those environmental factors affecting scallops though water temperature is considered most important. Past explorations have shown evidence of occasional mass mortalities that may have been due to temperature fluctuation. Dickie and Medcof (1963) reported that mass mortalities often occur in sea scallops when water temperatures fluctuate rapidly. Further, temperature changes may indirectly cause death through dehabilitation, thereby rendering scallops highly susceptible to predation.

DISTRIBUTION AND ABUNDANCE

Calico scallops occurred in 13 to 37 fm with greatest concentrations between 19 and 30 fm (Figure 1).

Depth distributional differences north and south of the Cape were noted (Figure 7). Scallops south of the Cape were generally found shallower than north of the Cape. The reasons for this are unknown though the thermal structure may be different in these areas. Also the shelf is narrower with a steeper gradient south of the Cape, and available habitat is restricted. Optimum bottom may occur 4 to 5 fm shallower in that area.

A slight seasonal change in depth distribution occurred in both areas. Scallops were in slightly deeper water in winter than summer, this difference being less noticeable north of the Cape.

Yearly differences in bed distribution were noted during the survey. In 1967 beds were primarily located between 19 and 30 fm; however, in the fall of 1968 very few scallops occurred in that depth range and a developing bed was found at lat 29°10′ N in 15 to 17 fm. This bed extended northward beyond lat 29°25′ N.

Scallops were usually found in north-south windrows several hundred yards to a quarter mile long. Bed size varies but without a means

FIGURE 5.—Dredge catch of starfish aboard the RV *Oregon* taken during an exploratory fishing survey on the Cape Kennedy grounds.

of underwater observation there is no way of mapping beds definitively. A remote-controlled underwater assessment vehicle (RUFAS) has since been used to survey the Cape Kennedy grounds and preliminary data analysis verifies our findings (Cummins et al., see footnote 5).

Major abundance occurred at slightly different depth ranges north and south of the Cape (Figure 8). Seasonal changes, while not noticeable in these areas, did occur at some depth ranges. Abundance was highest in 21 to 23 fm south of the Cape and 24 to 27 fm north of the Cape. By August 1968, scallops were gone south of the Cape. North of the Cape the population diminished through October 1968 and had practically disappeared by December.

YIELD

Yield is presented as pounds of meat per drag. Preliminary calico scallop investigations had used bushels per drag as a yield criterion, but owing to differences in size, barnacle encrustation on the shell, and changes in meat condition, the meat yield per bushel was highly variable.

Variation in bushel yield due to size differences is obvious, but the effect of barnacle encrustation is more subtle. The sedentary nature

FIGURE 6.—Starfish (*Asterias*) observed attacking calico scallops during a submarine survey off Cape Kennedy in September 1969.

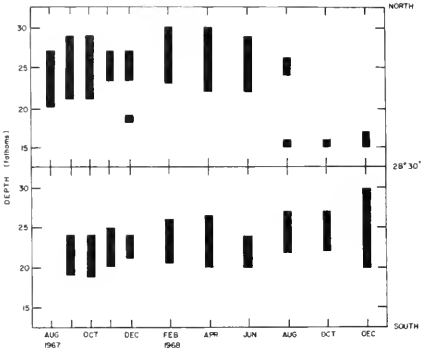

FIGURE 7.—Monthly depth distributions north and south of Cape Kennedy.

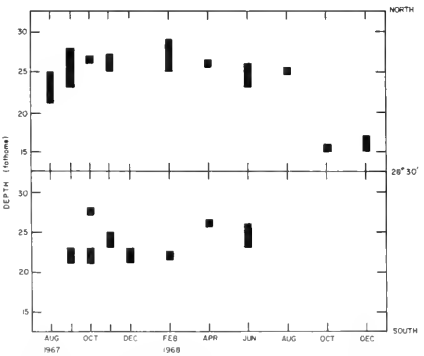

FIGURE 8.—Depths at which maximum abundance occurred north and south of Cape Kennedy.

of scallops enables a gradual buildup of barnacles on the shell which decreases the number of scallops per bushel. This leads to an overestimate of meat yield per bushel.

Meat condition was found to fluctuate seasonally. From December to April meat counts increased rapidly (Figure 9) owing to increased flaccidity resulting from physiological changes

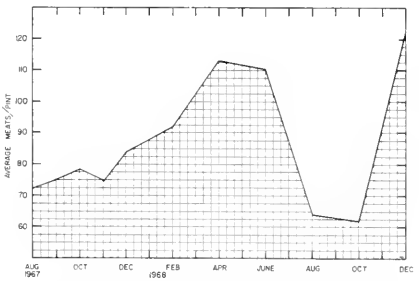

FIGURE 9.—Average number of scallop meats per pint by month during the 1967-68 Florida east coast survey.

associated with spawning. Though most scallops are generally large (50 to 60 mm) at spawning, the meat yield per bushel is much lower than during the fall when scallops are smaller.

These conditions caused us to discard number of bushels and use total pounds of meat per drag as a more meaningful measurement of yield.

Meat count per pint is an excellent index of fishing productivity when used in conjunction with total pounds. A wide seasonal variation in meat count was caused by differences in meat condition and scallop size (Figure 9). From August to December the meat count per pint stabilized at 70 to 80. During December through June the count increased due to deterioration of meat firmness and/or contribution by a nonspawning population remnant.

The fall increase in meat count per pint is due to increasingly large numbers of small scallops entering the fishery from the shallow bed on transect C.

Commercially significant yields were taken between September and February with a maximum in October (Figure 10). Yield rapidly decreased after February because of spawning. Yield in the fall of 1968 was appreciably lower than in 1967 owing to the failure of the survey to locate quantities of scallop. The population in 15 to 17 fm at lat 29°10′ N, spawned in 1968, was not found in any abundance during the Oc-

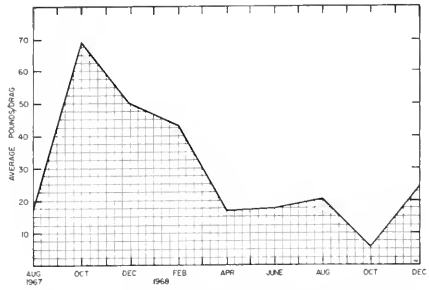

FIGURE 10.—Average number of pounds of scallop meats per 30-min drag by month during the 1967-68 Florida east coast survey.

tober or December cruises, indicating spawning occurred much later in 1968 than in 1967.

DISCUSSION

Reproduction in calico scallops is related to age rather than size. It is probably triggered by water temperature as Loosanoff and Davis (1950) have shown that raising the ambient water temperature induces spawning in some bivalves. Since spawning generally occurs in the spring, rising temperatures would be expected to be the initiating mechanism.

Maturation, taking 7 to 9 months beginning in late summer, is easily detected in the field by coloration changes in the ovaries. Resting ovaries are whitish-yellow but as maturation progresses their color changes through various stages of deepening yellow-oranges to a reddish-orange at the ripe condition. Ripe gonads have been collected from a wide size range indicating that the minimum age of sexual maturity is quite low. Data indicate maturation does not begin simultaneously in all scallops within even the same bed. This presumably accounts for the 2- to 3-month differential in maturation time.

Spawning begins in late February or early March and continues to June. In some areas the season is protracted since small scallops are caught throughout most of the year. This is probably due to variation in sexual maturation rate, growth rates, and water temperature. The lowest frequency of small scallops occurred in February indicating decreasing temperatures in the fall terminate any protracted spawning.

Protracted spawning during one season conceivably leads to protraction during the next. Since growth is related to temperature, scallops spawned early in spring have a longer growing season than do those spawned later in the year. Therefore, the older individuals in a year class would undoubtedly be larger at spawning than the younger individuals. Further, maturation is probably controlled by hormones which in turn are influenced by some environmental factor such as water temperature. This interaction between age, environment, and reproduction needs clarification.

Growth curves through all sizes ranges cannot be calculated from the survey data since the earliest stages are omitted. The curve is sigmoid from seed size to senility, rapidly increasing from 5 to 50 mm. Then the rate decreases through senility with maximum size about 80 mm.

Data from 1967 and 1968 indicate only one year class is present in spring but two year classes are present from early summer through winter. Therefore, the maximum age reached by calico scallops must not be greater than 24 months and averages 18 to 20 months.

Mortality rates computed from the survey data support a 2-year maximum life span. Post-spawning mortality averages 23% per month. At that rate only 20% of the year class would remain by early fall. Assuming that some mortality occurs during the pre-spawning period, the remaining population in 18 to 20 months would be exceedingly small.

Scallops are not randomly distributed but form north-south windrow configurations. These configurations, established at spat set, are heavily influenced by currents. Olsen (1955) indicates that windrow-like configurations result from strong tides, their orientation running lengthwise to the tide. He also shows strong linear configurations result in eddy systems.

This latter situation is analogous to that off eastern Florida. Little is known about substrate preference. Some beds offering optimum conditions may be perpetuated for several years, but competition for space between adults and spat must minimize such occurrences. If spat are unable to compete effectively for substrate, the presence of live adult scallops on the bed may prevent spat set. This question needs to be answered in the near future.

Yearly distribution and abundance depend on both spawning success and spat set. Commercial size and abundance are generally reached by early fall, usually in October. Fishing remains optimal until February when catch rates and yield decrease. This is due to spawning and associated factors such as increased mortality and meat deterioration.

In October 1967 scallops averaged about 45 mm and yielded 75 to 80 meats per pint. During October the survey produced an average catch of 70 lb. of meats per 30-min drag as compared with 20 lb/drag in April.

A fall and early winter fishery is expected to be most productive. Fishing is not recommended during spring because catch rates and yield are generally low and a spring fishery could have an adverse effect on the spawning population.

The nonrandom distribution and variable size of beds make it difficult to estimate the standing crop from dredge surveys. Scallops were found over approximately 285 square miles of bottom during the survey but less than 14% (37 miles) was covered by the dredge. About 6,500,000 scallops were caught during the survey. Scallops are not randomly distributed on the grounds but occur in beds where very high densities are often reached. Relatively few individuals occur between beds. Beds are difficult to measure but some were estimated to be several hundred yards in width and over a half mile in length.

Photographs taken during the *Aluminaut* dive show that densities of five scallops per square foot occur on some beds (Taylor, 1967). A minimum of 285 square miles of scallop bottom was found during the survey and varied densities occurred over that area. If 10% (28 square miles) supported densities approximating those found by the *Aluminaut*, then an estimated standing crop of 3,892,000,000 scallops existed in 1967-68. This figure is derived from the following: 28×27.8 million (no. ft^2/mile^2) $\times$ 5. That population could easily support 10 boats fishing at a rate of 1,500 lb/day. At an average of 70 scallops per pound, 10 boats would take a total of 31,500,000 scallops per month at the above rate. This is approximately 1% of the estimated standing crop.

Recent explorations with RUFAS indicate that densities estimated from the *Aluminaut* cruise may be ultraconservative (Cummins et al., see footnote 5). Films from the RUFAS survey indicate that scallops may reach densities as high as 10 or more per square foot providing a standing crop is considerably above that given previously. Findings from the RUFAS survey will be published in the near future by personnel from the Exploratory Fishing Station in Brunswick, Ga.

Yearly variations in distribution and abundance may at first glance be discouraging to a fishery. However, the remarkable opportunity to predict each fall fishery exists because distributional and abundance patterns are established in spring or early summer—perhaps as early as May or June. Population assessment at that time would provide estimates on the standing crop and determine the success of a fall fishery some 4 or 5 months prior to its onset. The authors tested this hypothesis in the spring and summer of 1970 using the RUFAS vehicle.

SUMMARY AND CONCLUSIONS

The survey data show that calico scallops have a short life span of 18 to 24 months. Spawning occurs after a 7- to 9-month sexual maturation period in early spring. Some protracted spawning was noted for localized areas. Although growth rates were not determined for sizes smaller than about 5 mm, the estimated growth curve between 5 mm and senility (75 to 80 mm) is sigmoid, rapidly increasing to about 50 mm and then decreasing to death.

Monthly mortalities for December to October average approximately 20%. Mortality curves were generally curvilinear after spawning, indicating a rapid post-spawning dieoff.

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EFFECTS OF DELAYED INITIAL FEEDING ON LARVAE OF THE GRUNION, *Leuresthes tenuis* (AYRES)

ROBERT C. MAY[1]

ABSTRACT

The initial feeding of newly hatched larvae of the grunion, *Leuresthes tenuis* (Ayres), was delayed for various periods of time under laboratory conditions at 18° C. Unfed larvae did not develop morphologically beyond the stage reached at the time of yolk absorption, about 4 days after hatching, although some survived as long as 3 weeks. Regardless of how long initial feeding was delayed, 80% or more of previously unfed larvae began feeding when food was made available to them, and at least 40% of the larvae alive when food was offered were able to survive to the end of a 20-day experiment. Some larvae feeding for the first time after 1 to 2 weeks without food, died after gorging themselves with *Artemia* nauplii. When food was offered to starved larvae, growth began and generally proceeded at about the same rate as in larvae fed from day 1, although there was some indication that a few days' delay in initial feeding increased the conversion efficiency of grunion larvae feeding on *Artemia* nauplii. Catabolism of fat provided most of the energy for metabolic processes during starvation. The condition factors and carbon/nitrogen ratios of unfed larvae were below those of fed larvae; condition factor seemed to be the better index of nutritional state. Grunion larvae probably do not experience high mortality at sea due to starvation, nor do they exhibit a classical "critical period" at the time of yolk absorption.

Most marine fishes pass through a free-swimming larvae stage, and it is well documented that survival through this stage is very low, generally being much less than 0.1% (e.g., Sette, 1943; Ahlstrom, 1954; Pearcy, 1962; Iizuka, 1966). The rate of survival through the larval stage is probably the most important factor determining the strength of year classes (Beverton, 1962; Gulland, 1965). Hjort (1914, 1926) advanced the hypothesis that larval survival was drastically affected by the abundance of food at the time the yolk was completely absorbed, and that poor year classes resulted when insufficient food was available to larvae at this "critical period." As Marr (1956) pointed out, Hjort's "critical period" concept has had a profound effect upon the thinking of fishery biologists.

Increased larval mortality at the time of yolk absorption has, however, proved difficult to demonstrate in nature. Marr (1956) concluded that the published evidence did not establish the existence of such increased mortality at sea; even in the light of more recent field data (Farris, 1961; Pearcy, 1962; Stevenson, 1962; Iizuka, 1966; Karlovac, 1967), it is difficult to decide from survival curves whether increased mortality at yolk absorption does in fact occur in nature. It has proved equally difficult to demonstrate that starvation is a major cause of larval mortality in the sea. Wild larvae found with empty guts (Lebour, 1920; Bowers and Williamson, 1951; Arthur, 1956; Bhattacharyya, 1957; Berner, 1959) may indicate imminent death by starvation or may reflect artifacts such as defecation or selective capture by plankton nets (Blaxter, 1965, 1969). Reports of apparently emaciated larvae, sometimes caught in regions where food is scarce (Soleim, 1942; Arthur, 1956; Shelbourne, 1957; Nakai, 1962; Hempel and Blaxter, 1963; Nakai et al., 1969), are suggestive but inconclusive (Marr, 1956; Blaxter, 1965, 1969). Field data thus indicate the possibility of high larval mortality due to starvation after the yolk has been absorbed but have not demonstrated its existence conclusively

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or determined its significance for year-class strength.

The response of larvae to food deprivation in the laboratory may provide badly needed evidence of how susceptible they are to starvation at sea. There have, however, been few attempts to determine experimentally the effects of delayed initial feeding on the larvae of marine fishes. Fabre-Domergue and Biétrix, the two pioneers of marine fish culture who coined the term "critical period," which Hjort later adopted, believed that feeding before the yolk supply was exhausted was essential to assure larval survival in laboratory rearing attempts. They stated that among larvae which received food prior to yolk absorption, ". . . *la phase que nous avions nommée période critique post-larvaire n'existe pas*" (Fabre-Domergue and Biétrix, 1898: 468). These authors went on to say that larvae which did not receive food early would subsequently become weak and unable to capture food and would exhibit considerable, if not total, mortality (Fabre-Domergue and Biétrix, 1898). The importance of early feeding for marine fish larvae was not further investigated in the laboratory until Blaxter and Hempel (1963) studied the effects of delayed initial feeding on the behavior of larval herring, *Clupea harengus* L. By feeding larvae after successively longer times without food, Blaxter and Hempel determined the time beyond which the larvae failed to exhibit feeding movements when supplied with food, a time they called the "point of no return." This point came 5 to 9 days after complete yolk absorption, at temperatures of 12° to 8° C, much later than the statements of Fabre-Domergue and Biétrix would have led one to expect. Recently, Lasker et al. (1970) observed the mortality of larvae of the northern anchovy, *Engraulis mordax* Girard, which had been fed at progressively later times after hatching. At temperatures of 15° to 22° C, larvae for which initial feeding had been delayed until 2.5 days after complete yolk absorption, showed the same pattern of mortality as groups of starved controls, while larvae receiving food 1.5 days after yolk absorption exhibited good survival—a phenomenon which these authors termed "irreversible starvation."

The purpose of the present study was to investigate in detail the changes which take place in starving larvae and in larvae whose initial feeding is delayed for various lengths of time, and thus to bring more evidence to bear upon the perennial questions of how susceptible larval fishes are to food deprivation and whether they do pass through a "critical period" at the time of yolk absorption. This study also sought to broaden the range of our knowledge of larval fish ecology by utilizing a species belonging to a group other than the Clupeiformes or Pleuronectiformes, or which nearly all of our information has hitherto been based. The fish chosen for study was the grunion, *Leuresthes tenuis* (Ayres), a member of the family Atherinidae. Atherinids produce rather large demersal eggs (Breder and Rosen, 1966), and the well-developed larvae which hatch provide an interesting contrast with flatfish and clupeoid larvae. Specifically, these experiments were designed to determine the effects of delayed initial feeding on mortality, on growth, and on the ability of grunion larvae to begin feeding and to utilize ingested food, and to ascertain what changes in the morphology and chemical composition of the larval body occur during starvation.

MATERIALS AND METHODS

SOURCE OF EGGS; HATCHING

The grunion is best known for its unusual habit of spawning on the sandy beaches of southern California and northern Baja California (Thompson and Thompson, 1919; Walker, 1952; Breder and Rosen, 1966). The eggs are deposited in the sand at night at certain times in the tidal cycle and are washed free some days later by a succeeding high tide, at which time the larvae hatch if the developmental period has been sufficiently long. The spawning season extends from late February or early March to late August or early September, with spawning intensity reaching a peak in April and May (Walker, 1952). During this time it is relatively easy to collect grunion eggs, which therefore provide

convenient material for the study of embryonic and larval development.

Eggs for the present study were collected during a spawning run on the night of March 24, 1970, at the beach in front of Scripps Institution of Oceanography, La Jolla, Calif. The eggs from a running ripe female were expressed into a small plastic container and artificially fertilized by adding milt from one male and a small amount of seawater; after approximately 1 min the water was decanted and sperm removed with several washes of fresh seawater. At the National Marine Fisheries Service Fishery-Oceanography Center in La Jolla, developing eggs were dispersed in a layer of washed, slightly moist beach sand approximately 1 cm deep at the bottom of rectangular plastic containers (16 × 12 × 11 cm), and a paper towel moistened with seawater was placed on the surface of the sand. The tops of the containers were covered with aluminum foil. This incubation procedure, essentially the same as one described by Morris (1956), kept the eggs moist and produced good hatching when excess water (which quickly brought on anoxic conditions) was avoided. The containers were placed in a water bath held at 20° ± 1° C by manually mixing water from the warm and cold seawater systems of the Fishery-Oceanography Center (see Lasker and Vlymen, 1969). The day before hatching, the temperature of the water bath was lowered to 18° C over a period of 3 hr.

On April 3, 1970, after 10 days of incubation (a common incubation time in nature, according to Walker, 1952), hatching was induced by adding filtered seawater at 18° C to the incubation containers and agitating the water and sand by drawing water rapidly in and out of a pipette. Hatched larvae were immediately transferred via pipette (4-mm bore) to 18° C water in rearing containers. In this paper the day of hatching will be referred to as day 0, the day after as day 1, and so forth.

DESIGN OF EXPERIMENT

A total of 20 rearing containers was set up, each with approximately 50 newly hatched grunion larvae. Seven containers (#1-7) were used to determine the effect of delayed initial feeding on mortality and growth; in six containers in this group, feeding was begun at progressively later times at 3-day intervals beginning on day 1—i.e., on days 1, 4, 7, 10, 13, and 16—while the larvae in one container (#7) were not fed and served as a control. On the 20th day after hatching, all surviving larvae which had received food in this series were collected, and the length, weight, and chemical composition of the larvae were determined. Seven of the other containers (#8-14) were fed daily beginning on day 1, and six (#15-20) were given no food; these containers, referred to as "supply containers" in what follows, supplied fed and unfed larvae for measurements of length, weight, and chemical composition and also for experiments on feeding and growth. On the same days when feeding was begun in a new container in the delayed feeding series (containers #1-7), larvae from both the "fed" and the "unfed" supply containers were used to determine the incidence of feeding and to begin quantitative feeding experiments.

PHYSICAL CONDITIONS

Water of approximately 33 ‰ salinity was taken from the seawater system of the Fishery-Oceanography Center. The larval fish containers were originally filled with HA Millipore-filtered seawater, and at weekly intervals filtered water was added to the fed containers in order to replace water removed with uneaten food and fecal matter (see below), the volume added usually being between 1 and 2 liters. The temperature in the water bath was kept at 18° ± 1° C, and temperatures in the larval containers were within 0.5° C of the bath temperature. Eighteen degrees is the midpoint of the 14°-22° C range of water temperatures which occurs off La Jolla during the spawning season of the grunion (Reid et al., 1958). Banks of two 40-watt "daylight" fluorescent lamps positioned 76 cm above the surface of the water illuminated the containers for 12 hr each day. The lights were timed to go on after sunrise, so that diffuse light entering through windows increased

slowly in intensity as the sun rose and no abrupt dark-light transition was imposed upon the larvae.

CONTAINERS

The containers used for rearing larvae in this study were the same as those described by Lasker et al., 1970), being circular (35 cm diameter, 14 cm deep) and made of the plastic alloy Kydex.[2] These containers have nonglossy black surfaces and hold 10 liters of seawater. For feeding studies with individual larvae, smaller Kydex containers were used (10.5 cm diameter, 4.2 cm deep) which held 300 ml of water. Both large and small containers were covered with clear plexiglass tops to reduce evaporation and keep out dust particles.

FEEDING

Nauplii of the brine shrimp, *Artemia salina*, were used exclusively as a food source. *Artemia* nauplii have proven to be poor food for larval clupeids but excellent for several other types of larvae (May, in press), and they appear to satisfy the nutritional requirements of larval grunion. Nauplii were obtained by hatching San Francisco brine shrimp eggs in trays modeled after those described by Riley (1966). Two trays were used, which allowed harvesting of trays on alternate days with a time lapse of 48 hr between inoculation of eggs and harvesting of nauplii. The water in the trays was kept at about 20° C. Nauplii were rinsed in filtered seawater and added to rearing containers shortly after the lights went on each morning, and more were added during the day if the concentration dropped low enough to prevent *ad libitum* feeding. If any uneaten nauplii remained from the previous day, as many as possible were removed by pipette before the morning addition of new nauplii. Fecal matter was siphoned daily from the bottoms of the "fed" containers.

[2] Kydex is manufactured by Rohm and Haas, Philadelphia, Penn. Use of trade name does not imply endorsement of the product.

QUANTITATIVE FEEDING STUDIES

At 3-day intervals beginning on day 1, 6-day quantitative feeding experiments were begun to measure the food consumption and growth of previously fed and unfed larvae. Larvae were transferred individually, from both the "fed" and the "unfed" supply containers, to small (300 ml) containers late in the afternoon on the day before the beginning of the feeding experiment. Three larvae from a "fed" container and three from an "unfed" container were used in each feeding experiment; individual larvae were kept in separate containers during the feeding study.

On the morning following transfer to the feeding containers, and for six mornings thereafter, a known number of *Artemia* nauplii was counted out with a pipette under a dissecting microscope and added to each container. Shortly before the lights went off at the end of the day, the grunion larvae were transferred by pipette to new containers, and the uneaten *Artemia* in the old containers were concentrated on a nylon mesh, preserved in Formalin and later counted. The difference between the number of nauplii added in the morning and the number left at the end of the day gave the number eaten by a larva. At the start of the series of feeding experiments, on day 1, 100 nauplii were added to each experimental container; when larvae consumed 70% or more of the nauplii offered, the number offered the following day was increased by 50 nauplii. On the morning following the final (6th) day of feeding, the experimental larvae were collected and analyzed as described below. The weight of a larva at the start of the feeding experiment, estimated from the mean weight of 10 larvae sampled at that time, was subtracted from the weight of the experimental larva at the end of the feeding period to yield its gain in dry weight.

In order to determine the weight of the ingested material, the weight of a single *Artemia* nauplius was estimated by making several weighings on an electrobalance[3] of groups of 5 to 20 nauplii, collected at the same interval after inoculation of eggs as the nauplii used in the feeding study. The nauplii were rinsed with distilled water and dried to constant weight at

60° C before weighing. The mean weight per nauplius was 1.64 μg, almost identical to Paffenhöfer's (1967) value of 1.65 μg per newly hatched *Artemia* nauplius. Where the larvae had yolk sacs at the beginning of the feeding period, the mean weight of the yolk masses dissected off the 10 larvae sampled at the start of the experiment (the dissection technique is described below) was added to the weight of the nauplii consumed to yield the total dry weight of the consumed material.

The growth and food consumption of individual larvae during the feeding period were thus known and allowed calculation of the efficiency of food conversion:

$$\text{percent conversion efficiency} = \frac{\text{dry weight gained}}{\text{dry weight consumed}} \times 100.$$

INCIDENCE OF FEEDING

The percentage of larvae which fed after progressively longer times without food, termed here the incidence of feeding, was determined in separate experiments in 10-liter containers. Approximately 25 larvae were transferred from one of the "unfed" supply containers to a 10-liter container with filtered seawater late in the afternoon preceding the experiment, and on the following morning large numbers of *Artemia* nauplii were introduced into the container. One hour later, the anesthetic MS-222 (tricaine methanesulfonate) was added to the container to a concentration of 132 mg/liter, and the anesthetized larvae were examined under a dissecting microscope for the presence of an orange-colored gut indicative of feeding on *Artemia* nauplii. Simultaneously, the incidence of feeding among larvae from one of the "fed" supply containers was determined in the same way, to serve as a control with which hitherto unfed larvae could be compared. Experiments of this sort were conducted on the same days on which food intake and conversion experiments were started, beginning with day 4. Owing to mortality from starvation, the numbers of larvae available in the "unfed" containers dwindled so that only 13 larvae were available for the feeding incidence experiment on day 13 and 4 on day 16. By day 16, body pigmentation had developed in larvae from the "fed" containers to such an extent that feeding incidence could not be assessed by examining the coloration of the gut, and no value was obtained for previously fed larvae on this day.

Anesthesis stimulated peristalsis in grunion larvae, as Blaxter (1965) observed in larval herring, but the procedure in the present experiment was rapid enough that at most only the contents of the rectum were being extruded during examination and all larvae which had in fact fed were recorded as such. It should be pointed out that, unlike the straight, tubelike gut of clupeid larvae, the gut of larval grunion is already differentiated at hatching into three more or less distinct portions, the last of which, the rectum, is separated from the rest of the gut by an "ileorectal valve" (Al-Hussaini, 1947) which inhibits rapid defecation of material not in the rectum.

MORTALITY

Dead larvae were removed from the containers each morning by pipette. A larva was considered dead when its brain had become opaque and it did not respond to water current or to tactile stimulation. Dead larvae were routinely examined with a dissecting microscope.

SAMPLING PROCEDURE AND ANALYSIS OF LARVAE

Larvae were collected by pipette, and their lengths measured with an ocular micrometer from snout to tip of notochord, or, after upward flexion of the tip of the notochord had taken place, to the posterior edge of the hypural elements (standard length). Only free-swimming larvae were sampled, although in "unfed" containers these became increasingly rare toward the end of the experiment. Sampled larvae were rinsed quickly in distilled water and placed on glass microscope slides. Since larvae which were sampled on days 1 and 4 still possessed yolk sacs, they were preserved in 3%

[3] Cahn Instrument Company, Paramount, Calif.

Formalin (in 50% seawater). Within 1 week of sampling they were rinsed in distilled water and their yolk dissected off, separated larvae and yolk masses being placed on microscope slides. The samples on slides were dried to constant weight at 60° C and weighed to the nearest microgram on an electrobalance. In agreement with the results of Blaxter and Hempel (1966), no effect of Formalin on the dry weight of larvae was found, nor was there a significant effect of Formalin on the dry weight of yolk masses (this was tested in a previous experiment by comparing dry weights of yolk dissected from preserved larvae with yolk dissected from frozen larvae or collected in preweighed capillary tubes).

Larvae sampled from the supply containers at 3-day intervals, and those collected from the delayed-feeding series on day 20, were analyzed for their ash, carbon, hydrogen, and nitrogen content. Percent ash was determined by weighing separately three randomly chosen larvae from each sample before and after combustion at 500° to 520° C. In the case of larvae fed from day 16 and sampled on day 20, only one larva was available for the ash determination. During combustion, larvae were held on tarred pieces of precombusted aluminum foil, and weighings were made on an electrobalance. The remaining larvae in each sample were ground into fine particles with an agate mortar and pestle, and two aliquots of this material from each sample were analyzed for carbon, hydrogen, and nitrogen content with a Model 185 carbon-hydrogen-nitrogen analyzer.[1] The number of replicates was limited by the amount of material available, but variation between replicate determinations was small, and means calculated from the replicates were accepted as the ash, C, H, and N values for the sample. This approach to the chemical analysis of larvae was chosen because it allowed determination of C N ratios, estimation of protein and fat content, and calculation of caloric content (see Results section).

Larvae which had been used in the feeding incidence experiments described above were preserved in 3% Formalin and later cleared in KOH and stained with Alizarin Red-S, the standard stain for bone, to allow comparison of ossification in fed and unfed larvae.

RESULTS

BEHAVIOR

Newly hatched grunion larvae have functional eyes and jaws and are extremely active (Thompson and Thompson, 1919; David, 1939). Grunion larvae which received food in the present experiment remained very active as they grew, and some schooling behavior was noted as early as day 6. Of more immediate concern was the behavior of starved larvae. On day 7 it was noted that unfed larvae were much easier to catch with a pipette than fed larvae. As the period of starvation lengthened, larval activity declined and the number of larvae remaining quiescent on the bottom increased. Near the end of the experiment, no starved larvae were swimming freely above the bottom, and their activity consisted in occasional erratic movements, followed by long quiescent periods.

SURVIVAL

Figure 1 shows the survival to day 20 of larvae which were fed at various times after

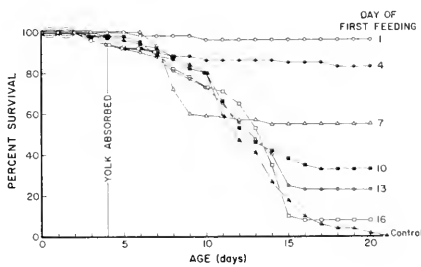

FIGURE 1.—Survival curves for larvae with different times of initial feeding, at 18° C. The number at the end of each curve indicates the day of initial feeding. The control group was given no food during the experiment.

[1] Hewlett Packard Corporation, Palo Alto, Calif.

hatching and a starved control. Yolk was completely used up in unfed larvae by day 4, and on this day only a minute amount was left in fed larvae. The survival curve for unfed larvae passed the 50% line between days 11 and 12, roughly the same as the starvation time given by Hubbs (1965) for larval grunion at 18° C. The starved control larvae were all dead by day 21. There is a direct relationship between percent survival of original larvae to day 20 and the day of first feeding (Table 1). The number

TABLE 1.—Survival to day 20 of larvae with different times of initial feeding.

Day of first feeding	Original number of larvae	Number of larvae alive when food first offered	Number of larvae alive on day 20	Percent survival to day 20	
				Original larvae	Larvae alive when food first offered
1	51	51	49	96.1	96.1
4	52	51	43	82.7	84.3
7	53	49	29	54.7	59.2
10	55	44	18	32.7	40.9
13	48	23	11	22.9	47.8
16	51	4	4	7.8	100.0

of larvae which survived to day 20, expressed as a percentage of those which were alive when food was first offered, is also listed in Table 1. This figure never dropped below 40%, and all of the previously unfed larvae alive in container #6 on day 16 (i.e., four larvae) began feeding when food was supplied and survived to day 20, and their general appearance and behavior indicated that they would easily have survived longer, had the experiment been prolonged.

In the group of larvae which was fed for the first time on day 7, 11 larvae were found dead on the morning following the day of first feeding; 9 dead larvae had food in their guts and 5 of these had guts which were bright orange and so stuffed with *Artemia* nauplii that the abdomen was noticeably distended. In the group fed initially on day 10, all eight larvae found dead the next morning had food in their guts, and four of these had bright orange, packed guts. On day 14, only one out of four dead larvae, found in the container which had been fed for the first time on the previous day, had food in its gut and showed the orange and bulging abdomen noted in dead larvae from the previous two groups.

GROWTH

The increase in length and dry weight of larvae sampled from the "fed" supply containers is presented in Table 2. The rate of growth was higher from day 16 on; variability likewise increased after this time, an example of the "growth depensation" which is commonly found in growing fish (Ricker, 1958). On day 7, the hypural elements were beginning to form along the posterior ventral margin of the notochord, and on day 10 the tip of the notochord was beginning its upward flexion. The greatest increase in length occurred between days 1 and 4, and owing to the upturning notochord the mean length actually decreased between days 13 and 16 (Table 2). On day 4, only the cleithrum and a very few cranial and branchial elements were ossified, but by day 10 about half of the vertebrae (the anterior ones) and some of the caudal rays were beginning to take up alizarin, and by day 16 all vertebrae and hypural elements were ossified.

TABLE 2.—Length and weight of fed and unfed larvae. $\bar{x}$ = mean, SD = standard deviation, n = number of larvae measured.

Age (days)	Length (mm)						Dry weight (mg)					
	Fed			Unfed			Fed			Unfed		
	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n
1	--	--	--	8.96	0.17	9	--	--	--	0.362	0.021	9
4	9.64	0.34	10	9.31	0.20	10	0.428	0.038	10	0.386	0.030	10
7	10.70	0.59	10	9.27	0.12	10	0.771	0.130	10	0.409	0.015	9
10	11.66	0.28	10	9.11	0.23	10	1.027	0.100	10	0.355	0.032	10
13	12.28	0.34	10	8.98	0.19	10	1.340	0.183	10	0.311	0.013	10
16	12.22	0.60	10	8.78	0.19	10	1.517	0.361	10	0.266	0.018	10
19	13.53	0.58	10	--	--	--	2.433	0.458	10	--	--	--
25	15.12	0.45	10	--	--	--	3.804	0.464	10	--	--	--

Starved larvae exhibited a slow decline in dry weight after yolk absorption, with little variability between larvae (Table 2). Although rudiments of the hypural elements were just discernible in starved larvae on day 7, their notochords never showed evidence of upward flexion, even as late as day 16. On day 16, ossification in starved larvae was comparable to that in fed larvae from day 4 or day 7, with only the cleithrum and a few elements of the cranium and visceral skeleton taking up alizarin.

The lengths and weights of 20-day-old larvae from the delayed feeding series (containers #1-6) are listed in Table 3, and in Figure 2 the weights are plotted and connected by dotted lines to the weights of starved larvae on the days when food was first offered. As expected, the later the initial feeding, the lower the mean weight on day 20, although the range of weights of larvae fed from day 4 falls within that of larvae fed from day 1, and larvae fed from days 13 and 16 likewise overlap (Figure 2). The rate of gain in weight decreases with delay of initial feeding, as indicated by the decreasing slopes of the dotted lines in Figure 2, but these rates are similar to those of larvae feeding for comparable lengths of time from day 1 (e.g., the weight gain of fed larvae between days 1 and 10 is about the same as that of larvae fed initially on day 10 and sampled on day 20). Larvae fed from days 1, 4, and 7 had completed notochordal flexion by day 20, and those fed from day 10 were at an intermediate stage of flexion at this time; in those fed from days 13 and 16, the process of flexion had not yet begun by day 20. Larvae from both the fed series and the delayed-feeding series indicated that notochordal flexion did not begin until a length of about 11 mm and a dry weight of about 1 mg had been reached.

TABLE 3.—Length and weight of 20-day-old larvae with different times of initial feeding. $\bar{x}$ = mean, SD = standard deviation, n = number of larvae measured.

Day of initial feeding	Length (mm)			Dry weight (mg)		
	$\bar{x}$	SD	n	$\bar{x}$	SD	n
1	14.24	0.61	20	2.702	0.420	20
4	14.03	0.36	20	2.513	0.193	20
7	12.38	0.31	20	1.638	0.148	20
10	11.10	0.38	18	0.995	0.086	18
13	9.87	0.51	11	0.561	0.137	11
16	9.82	0.35	4	0.436	0.036	4

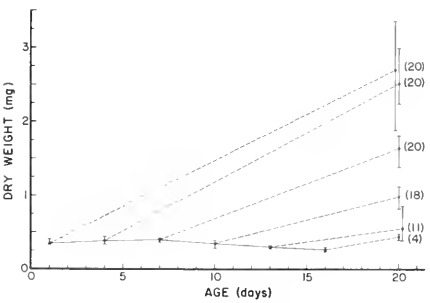

FIGURE 2.—Dry weights of 20-day-old larvae with different times of initial feeding. Means and ranges are plotted, and the number of 20-day-old larvae measured in each group is given in parentheses. Dashed lines connect mean weights at day 20 with the mean weights of unfed larvae on the days when feeding was initiated.

Condition factors have been used in the past to assess the nutritional state of fish larvae (Hempel and Blaxter, 1963; Blaxter, 1965) and were calculated for each sample in the present experiment as

$$\frac{\text{(mean dry weight, mg)}}{\text{(mean standard length, mm)}} \times 10^3$$

Figure 3A shows that after day 4, the condition factors for fed larvae increased until the end of the experiment on day 25, while those for starved larvae, after showing a slight rise on day 7, decreased until the final sampling on day 16. As shown in Figure 4A, the condition factors of 20-day-old larvae decreased in groups for which initial feeding had been delayed 7 days or more, with larvae fed from day 16 showing a condition factor between those of starved larvae 10 and 13 days old.

FEEDING INCIDENCE

On day 4, larvae which had been offered food since day 1 showed a higher feeding incidence (88%) than those which were given food for

the first time on this day (35%)—Table 4. However, on days 7, 10, and 13 the feeding incidence was similar (between 80 and 96%) in larvae which had fed and those which had not fed prior to the test. On day 16, the darkly pigmented abdomen of previously fed larvae made it impossible to determine their feeding incidence, but all of those larvae tested which had received no food prior to day 16 did consume food on this day. The failure of previously fed larvae to show a feeding incidence of 100% in these experiments probably reflects the stress associated with transfer between containers.

TABLE 4.—Feeding incidence of previously fed and unfed larvae. Larvae were exposed to *Artemia* nauplii for 1 hr, after which they were examined for evidence of feeding.

Age (days)	Larvae previously fed			Larvae previously unfed		
	Number of larvae feeding	Number of larvae not feeding	Percent feeding	Number of larvae feeding	Number of larvae not feeding	Percent feeding
4	22	3	88.0	9	17	34.6
7	21	2	91.3	23	1	95.8
10	22	2	91.7	24	1	96.0
13	20	4	83.3	8	2	80.0
16	--	--	--	4	0	100.0

FOOD INTAKE AND CONVERSION

On day 1, the mean dry weight of the larval yolk supply was 0.027 mg (range, 0.015-0.039 mg), and on day 4, fed larvae retained 0.003 mg of yolk (range, 0-0.011 mg) while starved larvae had no yolk left.

In the quantitative feeding experiments, conducted in small, 300-ml containers, some larvae did not survive the 6-day experimental period, and some exhibited erratic swimming behavior. Only data from the surviving larvae which displayed normal behavior have been retained. Larvae did not begin feeding until day 2, although food was available to them on day 1. The number of nauplii consumed daily per larva increased as larvae grew, from less than 50 in first-feeding larvae to almost 300 in larvae 2 weeks old and older. Table 5 gives the total food consumption, growth, and conversion efficiencies of all healthy larvae which survived the feeding experiments in small containers. Larvae which displayed growth comparable to that of larvae in 10-liter containers may be expected to give the most reliable conversion efficiency values and are identified by asterisks in Table 5. One larva, fed from day 1, showed the extremely high efficiency of 73%. Table 5 suggests a trend toward decreasing conversion efficiency as larvae get older. In the experiment begun on day 7, the previously unfed larva for which data are available showed a much higher efficiency than the previously fed larva.

BODY COMPOSITION

Results of the analyses of carbon, hydrogen, nitrogen, and ash in sampled larvae are given

TABLE 5.—Food consumption, growth, and conversion efficiencies of individual larvae in small containers during 6-day feeding experiments. Asterisks identify larvae which exhibited growth comparable to that of larvae in large containers and hence probably provide the most reliable conversion efficiency figures.

Age at start of feeding experiment (days)	Previous treatment	Dry weight (mg)				Percent conversion efficiency [= (gain/total food consumed) × 100]
		Total food consumed	Larva, initial	Larva, final	Larva, gain	
1	--	[1]0.433	0.362	0.678	0.316	73.0*
1	--	[1]0.771	0.362	0.700	0.338	43.8*
4	fed	[2]1.321	0.428	1.189	0.761	57.6*
4	unfed	1.074	0.386	0.880	0.494	46.0
7	fed	1.053	0.771	0.962	0.191	18.1
7	unfed	1.026	0.409	0.826	0.417	40.6
10	fed	2.250	1.027	1.940	0.913	40.6*
10	fed	1.640	1.027	1.528	0.501	30.5*
10	unfed	0.978	0.355	0.668	0.313	32.0
13	fed	2.000	1.340	1.657	0.317	15.9
16	fed	2.365	1.517	2.002	0.485	20.5
		2.155	1.517	2.418	0.901	41.8

[1] Includes 0.027 mg of yolk.
[2] Includes 0.003 mg of yolk.

TABLE 6.—Carbon, hydrogen, nitrogen, and ash in larval samples, as percentages of total dry weight.

Age (days)	Treatment	C (%)	H (%)	N (%)	Ash (%)
1	--	45.7	7.0	10.2	5.1
4	fed	45.5	7.0	10.5	5.4
7	fed	46.2	7.1	10.5	7.9
10	fed	47.1	7.1	10.8	8.6
13	fed	46.2	7.2	11.3	8.6
16	fed	45.2	7.0	10.9	8.7
19	fed	45.0	7.0	10.7	9.2
25	fed	43.1	6.6	10.9	10.0
4	unfed	46.5	6.9	10.4	6.6
7	unfed	44.5	6.9	10.6	7.8
10	unfed	44.0	6.8	11.1	9.4
13	unfed	44.0	6.8	11.2	9.2
16	unfed	43.2	6.4	11.3	7.3
20	fed from day 1	45.0	6.7	11.3	9.9
20	fed from day 4	43.1	6.7	11.1	9.8
20	fed from day 7	44.8	6.8	11.4	9.4
20	fed from day 10	44.8	6.7	10.8	8.2
20	fed from day 13	44.6	6.7	10.9	8.8
20	fed from day 16	43.3	6.5	10.6	11.4

in Table 6. In this paper the term *level* will be used in the sense of Giese (1969) to denote the percentage of the total dry weight which a particular body component constitutes. In fed larvae, the level of ash increased from 5.1% on day 1 to 10.0% on day 25. The ash level was higher in unfed than in fed larvae, except on days 7 and 16. The nitrogen level increased with age in both fed and starved larvae, but the increase was more steady in the latter. Fed larvae fluctuated between 10.7 and 11.3% nitrogen from day 10 to day 25. Accompanying the increase in nitrogen was a decrease in the level of carbon. Among 20-day-old larvae, the level of ash was higher, nitrogen lower, and carbon the same or slightly lower in larvae whose initial feeding had been delayed for 16 days than in larvae fed earlier. Nitrogen in 20-day-old larvae decreased with time of first feeding, from day 7 to day 16. The ratio of carbon to nitrogen has been plotted in Figures 3 and 4 along with condition factors. The C/N ratio is lower in starved than in fed larvae after day 4 but shows a decreasing trend with time even in fed larvae whose condition factor is increasing (Figure 3). On day 20, larvae whose initial feeding had been delayed 10 or more days had higher C/N values than larvae fed earlier; here too, decreasing condition factors accompanied increasing C/N values. Since preservation in Formalin has been shown to affect the C, H, N, and ash levels of copepods

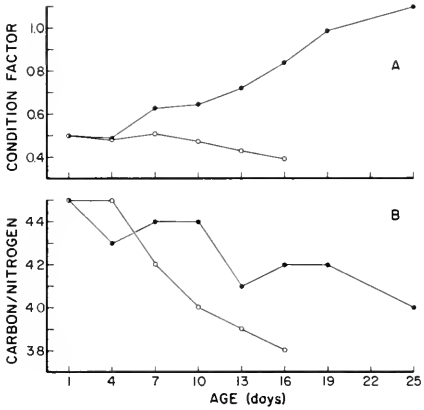

FIGURE 3.—Condition factors and carbon/nitrogen ratios of fed and unfed larvae. Condition factors were calculated as [(mean dry weight, mg)/(mean standard length, mm)] $\times 10^3$. Closed circles = fed larvae, open circles = unfed larvae.

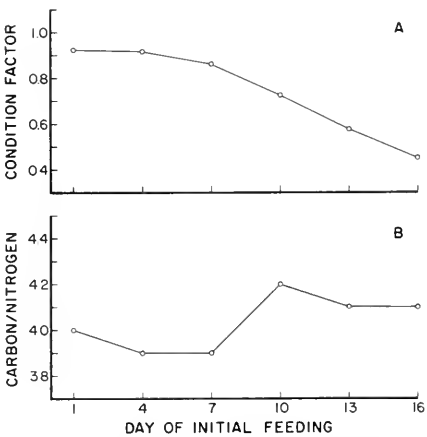

FIGURE 4.—Condition factors and carbon/nitrogen ratios of 20-day-old larvae with different times of initial feeding. Condition factors calculated as in Figure 3.

(Omori, 1970), the values given here for larvae 1 and 4 days old, which had been preserved in Formalin to allow removal of yolk by dissection, may be somewhat in error.

The level of protein in larval samples was estimated by multiplying the nitrogen level by 6.25 (White, Handler, and Smith, 1968), and fat was calculated by difference: 100 - (percent ash + percent protein) = percent fat. Nonprotein nitrogen and carbohydrate were assumed to be present in negligible amounts in this material (Lasker, 1962). Caloric content was calculated by multiplying weights of fat and protein in average larvae by 9.5 cal/mg and 5.7 cal/mg, respectively (Brody, 1945; Kleiber, 1961). Table 7 lists the resulting values. The most notable trends are an increase in protein level and decrease in fat level, in both fed and unfed larvae (Table 7). Unfed larvae had lower fat and about the same or somewhat higher protein levels than fed larvae. Among 20-day-old larvae, those for which initial feeding had been delayed tended to have higher fat and lower protein levels than those fed early (Table 7).

TABLE 7.—Protein, fat, and caloric content of larval samples. Protein and fat are given as percentages of total dry weight. Protein was calculated from the nitrogen content of samples, fat by difference, and caloric content by multiplying weights of protein and fat by standard conversion factors.

Age (days)	Treatment	Protein (%)	Fat (%)	Caloric content	
				cal/mg of dry weight	cal/mg of ash-free dry weight
1	--	63.8	31.1	6 60	6.97
4	fed	65.6	29.0	6.50	6.86
7	fed	65.6	26.5	6.25	6.79
10	fed	67.5	23.9	6.12	6.70
13	fed	70.6	20 8	6.00	6.54
16	fed	68.1	23.2	6.11	6.68
19	fed	66.9	23.9	6.09	6.70
25	fed	68.1	21.9	5.97	6.64
4	unfed	65.0	28.4	6.43	6.89
7	unfed	66.3	25.9	6.26	6.79
10	unfed	69.4	21.2	5.94	6.55
13	unfed	70.0	20.8	5.98	6.60
16	unfed	70.6	22.1	6.13	6.60
20	fed from day 1	70.6	19.5	5.89	6 54
20	fed from day 4	69.4	20.8	5.94	6.59
20	fed from day 7	71.3	19.3	5.90	6.50
20	fed from day 10	67.5	24.3	6.16	6.71
20	fed from day 13	68.1	23.1	6.07	6 65
20	fed from day 16	66.3	22.3	5.89	6.64

Reflecting changes in proximate composition, the caloric content of larval tissue showed an early decrease and from day 10 on showed no increasing or decreasing trend, in both fed and unfed larvae (Table 7). From day 10 on, starved larvae tended to have a lower caloric content than fed larvae. The caloric contents of 20-day-old larvae showed no consistent trend with time of initial feeding (Table 7).

DISCUSSION

The developmental process requires a nutritional input to supply energy and raw materials. In larval grunion which receive no food, development does not progress beyond the stage reached when the yolk is absorbed, although the larvae survive well beyond this point. The process of ossification is halted and the upward flexion of the notochord does not take place in unfed larvae, while tissue resorption, supplying energy for metabolic processes during starvation, results in a slow decrease in larval mass. Fat seems to be utilized most during starvation. The amount of fat in an average starving larva decreases by 0.071 mg, or 0.689 cal, during 16 days of starvation, while protein decreases by only 0.043 mg, or 0.245 cal (Tables 2 and 7). The fat and protein levels of feeding larvae are not greatly different from those of starving larvae (Table 7), but in the former case the observed increase in protein level with time must be a consequence of rapid protein synthesis in the growing organism, whereas in the latter it reflects the utilization of the body's fat reserves.

When food is offered to unfed larvae, growth begins and proceeds at about the same rate as in larvae fed from day 1 (Figure 2). Weight and body composition at day 20 in larvae whose initial feeding was delayed for various periods is close to that of larvae fed for similar lengths of time from day 1 (Tables 2, 3, and 7), though fat is much more depleted in larvae fed for 4 and 7 days starting on days 16 and 13, respectively, than in larvae fed for 4 and 7 days starting on day 1. Larvae fed for a period of 16 days, from day 4 to day 20, gained more weight and had higher protein levels than larvae fed from days 1 to 16 (Tables 2, 3, and 7), suggesting that a few days' delay in initial feeding caused

an increase in conversion efficiency. A similar effect has been found in adult fish (Ivlev, 1939; Pandian, 1967). There is some indication in the results of the quantitative feeding experiments that larvae convert food more efficiently after 7 days without food than after 7 days of feeding (Table 5), but the data are too meager to justify any conclusion on this point.

Omori (1970) showed that copepods from areas poor in food tended to have lower C N ratios than copepods from rich areas. In larval fishes, condition factors have been used in attempts to assess nutritional state (Hempel and Blaxter, 1963; Blaxter, 1965). Both measures were compared in the present study (Figures 3 and 4). Although starved larvae had lower C N ratios than fed larvae after day 4, due presumably to catabolism of fat, the C N ratios of growing, fed larvae decreased with age as a consequence of the rapid elaboration of protein, while their condition factors increased. Reflecting this same tendency, larvae 20 days old had higher condition factors but lower C N ratios, the longer they had been feeding. Condition factor seems to be somewhat more consistent and reliable as an index of the nutritional state of larval grunion than C N ratio.

In sum, larval grunion appear to be extremely resistant to food deprivation. Under laboratory conditions it takes 3 weeks for all larvae to die of starvation at 18° C (Figure 1). No matter how long initial feeding is delayed, over 40% of the larvae alive when food is offered will survive, and all larvae which survive 16 days without food can commence feeding at this time and survive (Table 1). Since grunion larvae hatch from eggs deposited in the beaches of southern California and northern Baja California and must inhabit inshore waters almost exclusively, and since the abundance of microplankton is extremely high in inshore as compared with offshore waters in this region (Beers and Stewart, 1967), it seems unlikely that these larvae ever experience high rates of mortality due to starvation. Major sources of mortality among grunion larvae must be sought, rather, in predation and physical damage from waves. Tidal variations may result in different incubation periods in grunion eggs from different spawnings (Walker, 1952), but the effect of this on larval viability has yet to be determined.

These findings differ from results for clupeoid larvae. In the northern anchovy a delay in initial feeding of 2.5 days after yolk absorption resulted in nearly complete mortality, even though many larvae were alive when food was administered (Lasker et al., 1970). This "point of irreversible starvation" appears not to exist for larval grunion, as starvation can in fact be reversed at any point along the survival curve of starved larvae (Figure 1).

Larvae of the herring (*Clupea harengus*) show a decrease in the percentage of larvae which commence feeding as the period of food deprivation is lengthened, and the point at which the percentage feeding is half that at the start of the experiment has been termed the "point of no return" (Blaxter and Hempel, 1963; Blaxter, 1965). Again, the grunion larvae show a different pattern, with at least 80% of the larvae commencing feeding when food is offered after periods of starvation ranging from 7 to 16 days (Table 4). Some larvae which did commence feeding after 7, 10, and 13 days without food were nevertheless unable to survive and died after gorging themselves with *Artemia* nauplii. The interesting fact that all of the larvae alive after 16 days of starvation commenced feeding and survived, while the percentage feeding was lower in larvae starved for shorter periods of time, may be explained as a result of mortality among the weakest larvae, so that by day 16 only the most hardy individuals were still alive.

Thus, certain types of larvae would be more likely than others to show a "critical period" pattern of mortality at sea under conditions of low food availability. If northern anchovy larvae were not to encounter food within 2.5 days after yolk absorption, there would ensue a catastrophic mortality concentrated in time (Lasker et al., 1970). In contrast, grunion larvae, which hatch in a more well-developed and robust state, would exhibit mortality extending over a number of days if deprived of food and hence would not show a "critical period" in the classical sense of Hjort. Obviously a sudden increase

in mortality at sea could come about after yolk absorption, or at any other time, owing to factors other than the availability of food. Hjort was, of course, not referring to larvae of the atherinid type when he enunciated his "critical period" hypothesis, but with the large volume of published material now available concerning the larvae of a few commercially important species, it would be easy to lose sight of the great diversity of larval forms and to apply ideas which may have validity in some groups to groups in which they have no place.

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THE RELATIVE SAMPLING PERFORMANCE OF 6- AND 10-FOOT ISAACS-KIDD MIDWATER TRAWLS

WILLIAM A. FRIEDL[1]

ABSTRACT

The relative abilities of 6- and 10-ft Isaacs-Kidd midwater trawls (IKMT) to sample macroplankton and fishes were assessed from comparable hauls taken with graded mesh nets during January and February 1967, in central Puget Sound. The plankton catch, mostly individuals 2 to 2.5 cm long, was dominated by the mysid, *Neomysis kadiakensis*. To quantify zooplankton data from the larger trawl, its cross-sectional area effective in filtering macroplankton was estimated for each month. The mean effective cross-sectional area of the 10-ft IKMT is 1.75 m^2. This implies a significant funneling of macroplankton by the forward section of the trawl.

The fishes taken were dominated numerically by Pacific herring, *Clupea harengus pallasi;* bay gobies, *Lepidogobius lepidus;* and plainfin midshipmen, *Porichthys notatus*. Herring was not taken by the 6-ft trawl; there was little apparent difference in the ability of the two trawls to capture midshipmen and gobies. Overall, the 10-ft IKMT caught more fish, more active fish, and larger fish than the 6-ft trawl. Though the 6-ft IKMT is probably adequate for studies with an emphasis on macroplankton, use of the 10-ft IKMT to sample fishes in inshore waters is preferable.

Interpretation of net haul data depends upon the capabilities and limitations of the sampling gear employed. With the plethora of equipment presently available for sampling the larger plankton and smaller nekton, comparative information on the relative sampling abilities of different gear is needed to equate results obtained with different nets.

This report deals with the relative sampling abilities of two sizes of Isaacs-Kidd midwater trawl (IKMT), a type of net widely employed in marine and freshwater investigations. The results apply to IKMT in general; the assessment elucidates the degree to which data obtained with different trawls are comparable.

METHODS AND MATERIALS

Samples were taken from the University of Washington 65-ft research vessel *Hoh* at night along a N-S track in Port Orchard, a narrow channel west of Bainbridge Island in central Puget Sound with a maximum depth of slightly over 40 m. Comparable IKMT hauls were made on two cruises in January and February 1967. Length frequency data are from five cruises made each month from November 1966 through March 1967.

Two sizes of IKMT with graded mesh nets were compared. The mouth area of the 10-ft IKMT (Figure 1) is 7.68 m^2 and that of the 6-ft IKMT (Aron, 1959) is 2.94 m^2. Mesh sizes for the various sections of the trawls and other

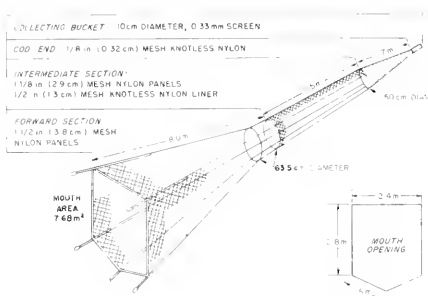

FIGURE 1.—Dimensions and construction details of the 10-ft Isaacs-Kidd midwater trawl used in this study (after Cooney, 1967).

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TABLE 1.—Dimensions and material specifications of the Isaacs-Kidd midwater trawls compared in this study.

Item	6-ft IKMT	10-ft IKMT
Mesh size		
Forward section	7.6 cm	3.8 cm
Intermediate section	1.3 cm	1.3 cm
Cod end	3.2 mm	3.2 mm
Cross-section area		
Mouth	2.94 m^2	7.68 m^2
Liner	1.26 m^2	0.32 m^2
Cod end	0.20 m^2	0.20 m^2
Filtering area		
Forward section	14.85 m^2	51.96 m^2
Intermediate section	7.02 m^2	9.81 m^2
Cod end	1.61 m^2	1.61 m^2

dimensional data are presented in Table 1. During each haul, speeds were measured at the surface with a Tsurami-Seiki-Koshakusho Co. flowmeter[2] while trawls were at depth. The same engine speed was used for all hauls. Generally, trawls were at depth 10 min and in the water less than 15 min total. In January, several 10-ft IKMT hauls were at depth 15 min. Net depth was monitored on deck from signals transmitted through the towing cable by a pressure-activated sensing unit (designed and built by the Department of Oceanography, University of Washington) mounted above the trawl. A Marine Advisers bathykymograph attached to the trawl bridle was read after each haul to check sampling depth.

The sampling distance was calculated from the speed and duration of each haul. This distance was multiplied by the appropriate trawl mouth area (Table 1) to determine the maximum volume of water filtered during each haul; the volumes so determined were used to calculate the monthly fish concentrations. For determinations of zooplankton concentrations, however, use of filtered volumes based on trawl mouth areas would result in concentrations inordinately low (Banse and Semon, 1963). Instead, volumes filtered should be based on the trawl cross-sectional area effective in sampling zooplankton of the size considered. The effective cross-sectional area, as used here, may be defined as that area which yields the correct zooplankton concentration, as measured independently, when divided into the zooplankton catch per unit distance of tow. Thus, if the length of tow is known, the number of animals caught per unit distance towed can be converted to concentration if the effective cross-sectional area is known. Banse and Semon (1963) compared euphausiid catches from a quantitative high speed catcher with those of the 6-ft IKMT and determined the effective cross-sectional area of the trawl to be not significantly different from the area of the opening of the middle (1.3-cm mesh liner) section of the trawl, namely 1.26 m^2 (Table 1). This effective area, multiplied by the sampling distance for 6-ft IKMT hauls, produced the effective volume of water filtered by the smaller trawl. Macroplankton concentrations were calculated for January and February from total 6-ft IKMT catch and total effective volumes filtered each month. Total 10-ft IKMT macroplankton catch was divided by the total sampling distance to determine the monthly macroplankton catch per kilometer by the larger trawl.

[2] The use of trade names is merely to facilitate descriptions; no endorsement is implied.

RESULTS AND DISCUSSION

Macroplankton samples from each trawl were compared to determine the effective cross-sectional area of the 10-ft IKMT. The mysid, *Neomysis kadiakensis*, represented 80% of the total catch; the mysid (*Acanthomysis macropsis*), the euphausiids (*Euphausia pacifica* and *Thysanoessa raschii*), and decapods of the genus *Crago* made up most of the rest. Most of the individuals were between 2 and 2.5 cm long. Plankton concentrations were considerably reduced in February (Table 2) and separate estimates of the 10-ft IKMT effective cross-sectional area were made for each month. Using the method of Banse and Semon (1963), division of the 10-ft IKMT catch per kilometer by the catch per 1000 m^3 filtered by the effective cross-sectional area of the smaller trawl yielded an estimate of the 10-ft IKMT effective cross-sectional area for each month (Table 2). The mean effective area, weighted according to the number of 10-ft IKMT hauls made each month, is 1.75 m^2 (Table 2). This is considerably larger than the area of the intermediate section open-

TABLE 2.—Summary of macroplankton catch data used to compute the 10-ft IKMT cross-sectional area effective in sampling macroplankton. Separate estimates were made for each month. The mean 10-ft IKMT effective area is computed from the monthly estimates and is weighed according to the number of hauls made with the larger trawl each month (see text).

Month	6-ft IKMT				10-ft IKMT				
	Hauls (no.)	Catch (no.)	Volume (10^3 m^3)	Concentration (catch/10^3 m^3)	Hauls (no.)	Catch (no.)	Distance (km)	Catch/km	Effective area (m^2)
Jan.	8	588	16.16	36.38	6	774	12.95	59.77	1.643
Feb.	8	128	17.41	7.35	12	221	16.62	13.30	1.810

Weighted mean effective area. $\frac{(1.643 \times 6) + (1.810 \times 12)}{18} = 1.754$ m^2

ing (0.32 m^2; Table 1) and indicative of a significant funneling by the forward section of the trawl.

The effective macroplankton sampling area of the 6-ft IKMT corresponds to the area of the opening of the intermediate section (1.3-cm mesh liner); effects of funneling by the forward section (7.6-cm mesh) of the trawl are not obvious (Banse and Semon, 1963). My results indicate that the forward section of the 10-ft IKMT, with 3.8-cm mesh, is relatively more important a factor in the ability of the net to sample macroplankton than the corresponding section of the smaller trawl. Given forward sections of the same mesh for both trawls, however, the effective macroplankton sampling area of the 6-ft IKMT would probably equal or exceed that of the 10-ft IKMT, for organisms of the size considered here. The ratio of the effective cross-sectional area to total trawl mouth area is 0.43 for the 6-ft IKMT and 0.23 for the 10-ft trawl. Thus, a larger percentage of the water entering the mouth of the smaller trawl is filtered for macroplankton. For this reason, and because the 6-ft IKMT is generally easier to handle and deploy, the smaller trawl would be preferred for studies with primary emphasis on macroplankton or small fishes.

The relative ability to each trawl to sample fishes was also assessed. Total catch figures are presented in Table 3. Numerically, Pacific herring, *Clupea harengus pallasi;* bay gobies, *Lepidogobius lepidus;* and plainfin midshipmen, *Porichthys notatus,* dominated the overall catch. Herring and gobies were common in hauls above 23 m while midshipmen were most abundant in deeper tows. A few shiner perch, *Cymatogaster aggregata,* Pacific cod, *Gadus macrocephalus,* spiny dogfish, *Squalus acanthias,* and miscellaneous flatfishes were also taken and are included in the category "Others" in Table 3. Though the total catch of the 10-ft IKMT exceeds that of the 6-ft IKMT for each category of fishes, the data are not directly comparable because the larger trawl filtered more water in each stratum. To equate catch data between trawls on the basis of equal volumes filtered, the 10-ft IKMT catch for each category of fish was multiplied by the ratio of 6-ft to 10-ft IKMT volumes filtered for each stratum (0.31 for hauls above 23 m; 0.47 for deeper hauls. Exact volumes given in Table 3). The product, expressed

TABLE 3.—Summary of fish catch data, by trawls, from hauls made in January and February 1967. Total catch figures are in whole numbers and concentrations of fishes (Individuals/1000 m^3 of water filtered) are in parentheses. Estimated 10-ft IKMT catch for volumes filtered equal to those of the 6-ft IKMT within each stratum is entered as "Equivalent 10-ft catch" and is directly comparable to the 6-ft IKMT catch data (see text).

Trawl	10 to 22 m						23 to 31 m					
	Hauls (no.)	Volume (10^3 m^3)	Herring	Bay gobies	Midship-men	Others	Hauls (no.)	Volume (10^3 m^3)	Herring	Bay gobies	Midship-men	Others
10-ft	14	171.4	27 (.16)	24 (.14)	4 (.02)	8 (.05)	4	53.7	1 (.02)	10 (.19)	48 (.89)	11 (.20)
6-ft	11	53.4	0 --	2 (.04)	0 --	1 (.02)	5	25.1	0 --	2 (.08)	22 (.88)	1 (.04)
Equivalent 10-ft catch			8	8	1	3			1	5	22	5

to the nearest whole number, is entered as "Equivalent 10-ft catch" in Table 3 and is directly comparable to the catch figures for the 6-ft IKMT in the row above it. Figures for the overall fish concentrations, from data on total catch and total volume filtered, are also given in Table 3.

When the catch data are compared on an equal volume filtered basis, the superior sampling ability of the 10-ft IKMT is evident. With the exception of *Porichthys* in the lower stratum, the 10-ft IKMT caught more fish of each category than did the 6-ft trawl and the resulting overall concentrations estimated by the 10-ft IKMT are likewise higher (Table 3). Pacific herring, a major component of the mid-depth sonic scattering layer in Port Orchard (Cooney, 1967; Friedl, 1970), were not taken by the 6-ft IKMT and apparently were capable of actively avoiding the smaller trawl. The less active, *Porichthys*, however, was sampled equally by the trawls in the lower stratum. Though the catch and concentrations of *Lepidogobius* appear much lower for the 6-ft IKMT in Table 3, the discrepancy may reflect gear selection and loss through the 7.6-cm mesh of the 6-ft IKMT forward section more than active avoidance of the trawl by the fish. The *Lepidogobius* captured were small, 35 to 50 mm SL, and probably were filtered only by the 1.3-cm mesh liner of the 6-ft IKMT intermediate section. Assuming all gobies taken by the 6-ft IKMT were filtered by the intermediate section only, the concentrations above and below 23 m would be 0.12 and 0.11 fish per 1000 m^3, respectively, and would more nearly approximate those of the larger trawl (Table 3). Thus, as with the macroplankton, the finer mesh of the forward section of the 10-ft IKMT enhanced the ability of the trawl to sample small organisms.

The 10-ft IKMT caught more fish and sampled active fish better than the 6-ft IKMT despite the fact it was generally towed at lower speeds (Table 4). The towing speed of the smaller trawl, though only slightly greater than that of the 10-ft IKMT, could have increased pressure waves and vibrations associated with the trawl and evoked greater avoidance responses

TABLE 4.—Net speed data for 6- and 10-foot IKMT hauls in January and February 1967. Speeds measured at the surface while the trawls were at depth. Average speeds are significantly different at the 99% level when compared with Student's t distribution (Simpson, Roe, and Lewontin, 1960).

Trawl	Hauls (no.)	Trawling speed (m/sec)		
		Range	Average	Confidence interval (95%)
10-ft	18	1.64 to 2.13	1.84	1.78 to 1.90
6-ft	16	1.99 to 2.25	2.14	2.09 to 2.19

in active fishes such as herring (Chapman, 1964; Harrisson, 1967). At present, knowledge of the pressure and vibration characteristics of IKMT underway is lacking and further conclusions regarding the influence of such characteristics on the sampling abilities of trawls would be speculative and beyond the scope of this paper.

Comparison of the length frequencies of *Porichthys* taken in deeper tows during the entire winter period (November - March) indicates a selection for larger fish by the larger trawl, despite its slower towing speeds and finer mesh (Figure 2). Nearly 70% of the *Porichthys* taken between 20 and 35 m by the 6-ft IKMT in the winter were less than 150 mm long (SL), while half the *Porichthys* taken by the 10-ft IKMT between 25 and 35 m in the same period exceeded 180 mm SL (Figure 2).

My results indicate the 10-ft IKMT catches more fish, more active fish, and larger fish than the 6-ft IKMT used in the study; similar conclusions, with respect to the ability of small and large trawls to sample mesopelagic fishes, were reached by Harrisson (1967). Aron and Collard (1969) studied the influence of net speed on catch for a 6-ft IKMT fully lined with 1.2-cm mesh netting and found that faster tows took larger fish of certain types off the California coast. My data, for inshore fishes, indicate mouth size, towing speed, net mesh size, and, perhaps, the dynamic characteristics of trawls combined with the behavioral aspects of the organisms sampled are all interrelated in a complex way to ultimately determine the sampling ability of a given trawl.

Standardization of gear and techniques used to sample midwater organisms would provide

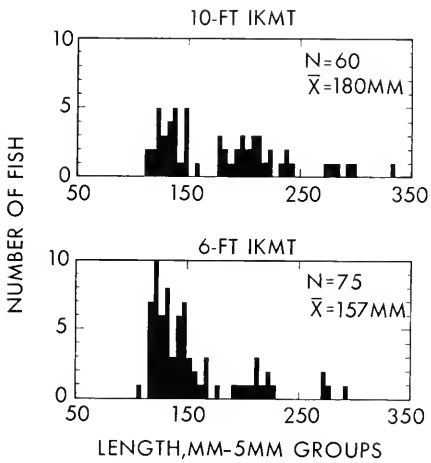

FIGURE 2.—*Porichthys notatus* length frequencies (SL) from hauls made on monthly cruises from November 1966 through March 1967. Ten-ft IKMT catch from five hauls between 25 and 35 m; volume filtered 57,400 m^3. Six-ft IKMT catch from 12 hauls between 20 and 35 m; volume filtered 58,000 m^3. Total catch (N) and mean length ($\bar{X}$) given on graphs for each trawl.

a more valid basis for comparison of different samples, but limitations of resources and equipment often determine the manner and means by which samples are taken. In this study, for instance, the strain of towing the 10-ft IKMT severely taxed the running rigging of the research vessel and prevented the use of the larger trawl on some cruises. At best, results of studies, such as this and that of Aron and Collard (1969), illuminate the limitations and capabilities of sampling gear, characteristics which must be recognized even with widely employed equipment such as the 6-ft IKMT. That trawls may sample only a limited portion of the fauna present is obvious and must be recognized. For instance, recent work in Puget Sound with large trawls indicates concentrations of herring, determined by IKMT hauls in winter sonic scattering layers, may be at least two orders of magnitude low (T. S. English, unpublished data). Thus, discussion of the biological "universe" defined by samples from a given trawl must acknowledge the limitations of the gear employed and avoid conclusions beyond the scope of the data available from the sampler.

In general, my results indicate the 10-ft IKMT to be preferable to the 6-ft IKMT for biological surveys emphasizing fishes in inshore waters, provided the vessel employed is capable of handling the large trawl on a regular basis. For surveys of macroplankton, however, the 6-ft IKMT is adequate and generally easier to deploy. The larger mouth opening and overall finer mesh of the forward section apparently enable the 10-ft IKMT to sample more fish, larger fish, and more active fish better than the 6-ft trawl. Fully lining the trawls with fine-mesh netting (UNESCO, 1968) would help simplify analysis of results by eliminating the need for estimating effective sampling cross-sectional areas when calculating concentrations of small fishes and macroplankton. Such lining would probably also increase the trawls' ability to sample smaller fishes (Backus et al., 1970), but the overall capabilities of the trawls to sample large or active forms would likely change little and utilization of the 10-ft IKMT would still be recommended.

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STUDIES ON THE USE OF CARBON DIOXIDE DISSOLVED IN REFRIGERATED BRINE FOR THE PRESERVATION OF WHOLE FISH

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ABSTRACT

Although storing fish in refrigerated seawater has many advantages over storing them in ice, the use of refrigerated seawater also has several disadvantages, one of which is the difficulty in controlling the growth of spoilage bacteria in the fish. Reported here is the effect on the growth of bacteria in rockfish and chum salmon of dissolving carbon dioxide in brine. Storing the fish in the refrigerated brine treated with carbon dioxide inhibited the growth of the bacteria, retarded the rate at which the fish decrease in quality, and increased their storage life by at least 1 week.

Refrigerated seawater as a medium for cooling, storing, and transporting fish has many advantages, which have been well documented (Idyll, Higman, and Siebenaler, 1952; Osterhaug, 1957; Cohen and Peters, 1962; Peters and Dassow, 1965; Roach et al., 1967).

This medium, however, also has disadvantages. These include the excessive uptake of water by species of low oil content, such as sole and cod, and an increase in total salt. Controlling the growth of spoilage bacteria in fish stored in refrigerated seawater also presents a problem (Roach et al., 1967). This problem results from the blood, dissolved protein, and visceral contents accumulated in the seawater during the storage of the fish. For these reasons, fish held in refrigerated seawater are not necessarily of better quality than are those held for the same period in ice. Nor can fish necessarily be held longer in refrigerated seawater than in ice before spoilage occurs.

This laboratory recently began a study of methods for increasing the effectiveness of refrigerated seawater as a medium for preserving fish. The investigation is timely because fishermen are finding it increasingly difficult to locate catches on traditional fishing grounds. This reduced abundance requires longer stays at sea, which sometimes result in the landing of fish of less than optimum quality.

Use of carbon dioxide gas dissolved in refrigerated seawater seemed promising as an inhibitor of the spoilage bacteria. Stansby and Griffiths (1935), for example, found that whole haddock and haddock fillets stored in an atmosphere of carbon doxide kept almost twice as long as did those stored in air. Castell (1953) demonstrated that carbon dioxide showed promise of being a useful preservative for salted fish held in 12% brine. Carbon dioxide has been used effectively to extend the storage life of refrigerated meat and poultry products (Wheaton, 1960) and is known to have bacterial inhibiting properties (King and Nagel, 1967). Fiskeriministeriets Forsogslaboratorium (1968) noted that, in limited experiments on holding fish in tanks, carbon dioxide decreased the rate at which their quality was degraded. Wayne I. Tretsven (1968, personal communication) showed that the shelf life of fresh silver salmon refrigerated in a mixed atmosphere of carbon dioxide, oxygen, and nitrogen was significantly extended beyond that of fresh silver salmon refrigerated in air.

Rockfish is normally iced aboard the fishing vessel and may be held for as long as 7 to 10 days before being landed. Chum salmon is frequently held in refrigerated brine aboard cannery tenders and may be held aboard the vessel for as long as 7 days. With both methods of holding, the quality of the fish may be poor if they must be held for longer periods. This

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study is specifically concerned with the effects that holding in modified refrigerated brine containing dissolved carbon dioxide[2] has on the storage life and quality of rockfish and chum salmon.

PRINCIPLES OF THE MODIFIED REFRIGERATED BRINE SYSTEM

EFFECTS OF DISSOLVING CO_2 IN REFRIGERATED BRINE

Carbon dioxide is a relatively inert chemical compound. It is almost odorless and, in the gaseous form, is colorless. Combined with water, it forms carbonic acid, a weak acid. Depending on the conditions, only part of the CO_2 added to the water is dissolved. The undissolved CO_2 either rises to the surface of the solution and is wasted away or else becomes suspended as gas bubbles, thereby forming carbonated water. The amount of CO_2 that can be dissolved by water depends on the pressure and temperature. The higher the pressure of the CO_2 and the lower the temperature of the water (at least, down to 35° F), the greater the amount of CO_2 dissolved. (We found that lowering the temperature below 35° F did not increase solubility.)

When chilled brine is saturated with CO_2, its pH is reduced from about 7.5 or higher to about 4.0. This change in pH from the alkaline to the acid condition helps to inhibit the growth of bacteria that contribute to spoilage (Wheaton, 1960). But pH control is not the only operative factor. Dissolved CO_2 seems to inhibit the metabolic processes of spoilage organisms and, of course, temperature control is important in slowing growth rate.

Although the modified refrigerated brine technique produces positive effects with regard to the control of bacteria, the addition of CO_2 can, under certain conditions, produce undesirable side effects. These effects are manifested in the form of accelerated corrosion rates of metals exposed to seawater containing high concentrations of the dissolved CO_2.

[2] In this report, the term "modified refrigerated brine" will henceforth mean brine containing dissolved carbon dioxide (CO_2).

REFRIGERATED BRINE EQUIPMENT

The equipment we used consisted of two fiber glass-insulated 55-gal epoxy-coated drums and a brine chiller.

We cooled the drums by circulating refrigerated brine from the brine chiller through 200 ft of ¾-inch polyvinyl chloride tubing wound around the outside of the drums in series and returned to the chiller (Figure 1).

Polyethylene liners with a capacity of about 30 gal were suspended in the drums by clamps. (The purpose of the liners was to keep the fish away from the cold sides of the drum, where they tend to freeze.)

Each drum was equipped with a Moyno[3] pump (Figure 2) for recirculating chilled brine (a solution containing 3.3% sodium chloride).[4] The brine was circulated by the pumps through a fitting in the bottom of the polyethylene liner. It was then forced through fish that had been placed in the liners, whereupon it overflowed back into the drums. The brine in the drums was picked up by a suction hose and recycled through the pumps at the rate of 10 gal/min. For maximum diffusion into the brine, the CO_2 gas was fed into the suction side of the circulating pump at the rate of 0.2 ft^3/hr. The brine in the other drum was left untreated for use as a control.

STORAGE LIFE AND QUALITY OF ROCKFISH HELD IN MODIFIED REFRIGERATED BRINE

OBJECTIVE MEASUREMENTS

Both bacteriological and chemical measurements were made. All measurements reported here were made in duplicate.

[3] The use of trade names is merely to simplify descriptions; no endorsement is implied.

[4] Sodium chloride brine was used in lieu of natural seawater because clean seawater was not convenient to the laboratory. However, this technique has previously been used by Collins (1950), Davis and Clark (1944), and others and found to give good results. In comparative experiments conducted by Roach and Harrison (1954) and more recently by this laboratory (unpublished), the test results showed that fish held in refrigerated brine were of equal quality to fish held in refrigerated seawater.

FIGURE 1.—Arrangement of the brine chiller (on the left); the pump is (on the floor) for circulating the chilled brine through the cooling coils shown wrapped around the uninsulated holding tanks.

Bacteriological Measurements

Materials and methods.—Described here are the rockfish and brine samples we used and the methods of making total plate counts.

The rockfish, *Sebastodes flavidus*, were caught in a trawl off the coast of Oregon. In the preparation of the samples, 130 lb. of the fresh, whole fish was divided into two equal lots. Each lot was placed in a polyethylene-lined drum of brine at a one-to-one ratio by weight of fish to brine. At this time, the iced fish had been out of the water 24 hr. The ratio by weight of fish-to-brine was maintained throughout the experiment by removing a known weight of brine at each sampling period.

One tank of brine was treated with CO_2 gas before the fish were loaded into it. The brine in both tanks was cooled to 31° ± 0.5° F during the experiment.

Periodically three fish and a sample of brine were removed from each of the storage drums for examination. The fish samples were used to make both the objective and subjective measurements at each sampling.

Total bacterial plate counts were made on the fish by the methods described by Pelroy and Eklund (1966). Briefly, the method was as follows: a slice of flesh was removed from near the dorsal side of each fish just posterior to the nape. Each subsequent experimental sampling was made from the same side and area of each fish tested. Forty-five grams of fish from the excised samples was homogenized aseptically

FIGURE 2.—Arrangement of the Moyno pump for recirculating chilled brine in the holding tank (on the left) and the CO_2 cylinder (in the back) and attached CO_2 flow meter (being adjusted by the worker).

with 180 ml of sterile 0.1% peptone solution at 38° F. Serial dilutions in 0.1% peptone-water were prepared for pour plates from the homogenate. Total plate counts were made by use of a TPY medium (0.5% yeast extract, 1.5% trypticase, 0.5% phytone, 0.2% glucose, 0.5% NaCl, and 1.5% agar). Counts were made on the brine by taking 1-ml samples, making appropriate serial dilutions in the 0.1% peptone-water mixture, and plating out onto the TPY medium. The plates were incubated at 22° C for 5 days.

Results and discussion.—Table 1 gives the results of the total-plate-count analyses on the brines and on the flesh of the rockfish. The data from the untreated brine show that a lag in bacterial growth occurred during the first 3 days of the storage test. After the third day, however, the population of bacteria in the brine increased rapidly.

Total plate counts made on the brine treated with CO_2 did not show a significant increase in the number of bacteria during the 17 days of storage.

Bacterial growth in the flesh of the rockfish held in the untreated brine was not inhibited during storage. On the 10th day, the fish were judged, by appearance and odor, to be inedible and unfit for testing. At this time, the total plate counts each exceeded a million organisms per gram of flesh. (A total plate count of a million organisms per gram usually indicates flesh of poor quality.)

TABLE 1.—Chemical and microbiological changes occurring in CO_2-treated refrigerated brine and in untreated refrigerated brine, and in the flesh of rockfish held in these brines.

Time in storage	Data on refrigerated brine with added CO_2							Data on refrigerated brine without CO_2				
	CO_2 conc.		pH		Salt conc	Total bacterial plate count		pH		Salt conc	Total bacterial plate count	
	Flesh	Brine	Flesh	Brine	Flesh	Flesh	Brine	Flesh	Brine	Flesh	Flesh	Brine
Days	*ppm*	*ppm*			*%*	*no./g*	*no./ml*			*%*	*no./g*	*no./ml*
0	119	1,000	6.7	4.0	0.2	1.2×10^4	1.3×10^4	6.7	6.8	0.2	1.2×10^4	1.3×10^4
3	562	2,332	5.8	5.3	0.5	--	1.6×10^4	6.4	6.9	0.6	7.7×10^4	1.2×10^4
8	842	1,848	6.4	6.0	1.1	1.6×10^4	--	6.5	7.3	1.0	2.4×10^6	2.8×10^6
10	--	--	--	6.0	1.3	--	--	--	--	--	Spoiled[1]	$>10^6$
14	597	1,452	6.5	6.0	--	1.4×10^4	5.3×10^5	--	--	--	--	--
17	--	--	6.4	6.1	1.8	2.0×10^4	3.8×10^4	--	--	--	--	--

[1] Samples judged by appearance and odor to be inedible and unfit for tasting.

The storage of rockfish in the refrigerated brine containing the CO_2 was terminated after 17 days. At this time, the results of the total plate counts made on the flesh of the fish showed that the microbial population had not changed significantly from the initial total plate count of 10^4 organisms per gram of flesh.

Chemical Measurements

pH.—The pH of the flesh of the rockfish and of the brines was measured by means of a Beckman combination electrode. The pH of the flesh was measured by inserting the tip of the electrode into the flesh (Patashnik, 1966).

Table 1 gives the results of the pH measurements of the fish and the brine. After 8 days of continuous recirculation, the pH of the control brine changed from a slightly acid condition (pH 6.8) to a slightly alkaline condition (pH 7.3). This change coincided with an increase in the microbial population in the control brine and was probably due to the formation of ammonia and amines from the bacterial degradation of proteins dissolved in the brine.

The initial pH of the brine treated with CO_2 shows the effect of the dissolved CO_2. The measurement was made before the fish were loaded into the brine. The subsequent increase in the pH of the brine in the presence of additional CO_2 may be attributed to the buffering by the soluble proteins in the blood and slime. After the 8th day of the experiment, the pH of the brine treated with CO_2 did not increase significantly.

Between the initial examination and that on the 3rd day, the pH of the flesh of the fish held in the brine treated with CO_2 dropped appreciably. This change was coupled with an increase in the concentration of the CO_2 in the flesh. As storage continued, the pH returned to the same level (6.4 to 6.5) as that of the flesh held in the untreated brine.

CO_2 concentration.—The concentration of CO_2 in the flesh and brine was measured by the method of Umbreit, Burris, and Stauffer (1957). The procedure was essentially as follows: A slice of fish was removed from the thickest part (dorsal side) of the fish. The sample was then carefully sectioned into horizontal cuts about ¼-inch thick and the individual cuts analyzed. A sample of the flesh or of brine was blended in Tris buffer (hydroxymethyl) aminoethane at a pH of about 9. Five grams of the mixture was added to a Warburg flask, and 0.7 ml of 0.5 M citrate buffer at pH 4.0 was added to the side arm of the flask. After the flask and its contents came to equilibrium at 38° F in a water bath, the contents of the side arm were tipped into the flask. The increase in manometric pressure was recorded at irregular intervals of time ranging up to 10 min. The amount of CO_2 evolved was calculated from a standard curve prepared by determining the changes in pressure after measured amounts of acid were tipped into known concentrations of bicarbonate.

Penetration studies carried out on whole rockfish showed that CO_2 diffused into the flesh very slowly. The maximum depth of penetration into the flesh was 0.75 inch. This depth was reached in about 8 days of storage. The highest

concentration of CO_2 in the flesh (842 ppm) was reached at this time.

The retention of CO_2 in the flesh was given consideration as a potential problem in contributing to an abnormal head-space pressure in canned salmon and to the separation of breading on breaded rockfish products. The initial indications were, however, that the retention of CO_2 will not be a problem. As was remarked earlier, CO_2 is not absorbed well at above-normal storage temperatures. CO_2 will therefore likely be dissipated from the flesh during routine cleaning, heading, and washing operations, which are done at temperatures considerably higher than those of storage. In an experiment in which red salmon were held in modified refrigerated natural seawater and commercially canned, no problems were encountered as the result of CO_2 retention. In canned products such as tuna and shrimp, the retention of CO_2 should not be a problem, because these products are exposed to relatively high preprocessing temperatures.

Additional consideration of these potential problems, however, will be given to the retention of CO_2 in future studies on modified refrigerated brine.

Salt concentration.—The concentration of sodium chloride was measured by the method described by Greig and Seagran (1965). In brief, a plastic-strip indicator containing a sensitized capillary element was placed in a filtered extract of fish and distilled water. After the reading was taken by means of the indicator, the concentration of salt in the extract was read from a standard curve supplied by the manufacturer of the device.

During the first 8 days of storage, the uptake of salt was similar in the fish held in treated brine to that in the fish held in the control brine. Concentrations of salt in the fish held in the untreated brine for longer than 8 days were not determined, because these fish spoiled at about that time. The fish held in the treated brine were analyzed for concentration of salt on the 10th and 17th days of storage. They showed somewhat more uptake of salt at each of these times.

SUBJECTIVE MEASUREMENTS

Raw Rockfish

At each sampling, a trained taste panel determined the effect of storage of the fish in two kinds of brine water. The fish were also evaluated in the round for general appearance and odor.

During the first 3 to 4 days of storage, fish held in either of the two brines were of good color, odor, and texture. By the 5th day, odors occurred in the fish held in the untreated brine. Between the 7th and 10th days, the fish were judged, by appearance and odor, to be unfit for tasting. During this time, the untreated brine had a strong odor of putrefaction and was dark brown.

The fish held in brine treated with CO_2 retained good color, odor, and texture for 17 days. The brine was almost colorless and odorless at the end of the experiment.

Cooked Rockfish

Cooked rockfish were prepared for taste-panel evaluation by the method of Miyauchi, Stoll, and Dassow (1964). The samples of cooked fish were evaluated for appearance, odor, flavor, texture, and overall quality, using a 10-point numerical scale.

Table 2 gives the sensory scores for the cooked samples. The data show that the fish in the untreated brine spoiled between the 7th and 10th days of storage. Except for an increase in saltiness, which occurred in the fish in either

TABLE 2.—Sensory evaluations on the cooked flesh of rockfish held in CO_2-treated refrigerated brine and untreated brine (control).

Time in storage	Overall sensory score[1]		Comments	
	Brine and CO_2	Brine (control)	Brine and CO_2	Brine (control)
days				
0	9.0	9.0	--	--
3	8.0	8.0	Slight salty taste	Slight salty taste
7	7.0	6.0	Unobjectionable salty taste	Off-odors
10	--	Spoiled	Unobjectionable firm texture	Foul off-odors
14	7.0	--	Firm texture	--
17	7.0	--	Objectionable salty taste; texture, color, and odor good	--

[1] A score of 10 denotes a product of highest quality, one of 5 denotes a product of borderline quality

of the two storage environments, the fish held in the modified brine water were organoleptically acceptable and of good quality after 17 days of storage. The subsequent refrigerated shelf life of this product was not determined.

STORAGE LIFE AND QUALITY OF CHUM SALMON HELD IN MODIFIED REFRIGERATED BRINE

OBJECTIVE MEASUREMENTS

Both bacteriological and chemical measurements were made. All the reported measurements were made in duplicate.

Bacteriological Measurements

Materials and methods.—Described here are the samples of salmon and of brine and the methods of making total plate counts.

About 300 lb. of fresh seine-caught chum salmon, *Oncorhynchus keta*, were obtained in the round from Bellingham, Wash. The salmon, which weighed about 10 to 13 lb. each, were divided into two lots of equal size. Each lot was loaded at a one-to-one brine-to-product ratio by weight into a drum of circulating brine containing 3.3% NaCl (see footnote 4). The salmon had been held in ice and, at this time, were less than 24 hr out of the water.

The brine was precooled and treated as was described in the section on rockfish.

Three salmon and a sample of brine were removed periodically for examination. The fish samples were used to make both the objective and subjective measurements at each sampling.

Total bacterial plate counts were made of the bacteria on the skin of the fish. Samples of the bacteria were obtained by the swab technique of Tretsven (1963). Briefly, the procedure consisted in swabbing the skin of the fish with a sterile swab through a 2 cm^2 hole cut into the center of a sterilized metal template. The tip of the swab was broken off in such a way that it fell into 10 ml of a 0.1% peptone solution, which was then mixed. Appropriate serial dilutions were made from this mixture and were plated out on the TPY medium (see the bacteriological section described under rockfish) for the determination of total bacterial counts.

In previous experiments at this laboratory, the swab technique gave results similar to those obtained from samples of flesh. Because of this finding and because of the relative simplicity of the swab technique, we used it in this experiment. Total plate counts of the bacteria in the brine were made by the method used in the rockfish experiment.

Results and discussion.—Table 3 shows the total plate counts made on the untreated and treated brines. In the control brine, the bacterial population steadily increased during the 18-day experiment. About the 7th day of storage, the brine evidenced a slight odor of spoilage. By the 11th day, the untreated brine smelled intensely putrid. At that time, the total plate count exceeded 10^6 organisms per milliliter.

The effect of CO_2 is demonstrated by the essentially unchanged bacterial population in the treated brine during the experiment. The bacterial population increased between the 3rd and 9th days but appeared then to stabilize. During the experiment, the brine remained odorless and,

TABLE 3.—Chemical and microbiological changes occurring in CO_2-treated refrigerated brine and in untreated refrigerated brine, and on the flesh of salmon held in these brines.

Time in storage	Data on refrigerated brine with added CO_2				Data on refrigerated brine without CO_2			
	pH of brine	Salt conc.	Total bacterial plate count		pH of brine	Salt conc.	Total bacterial plate count	
			Skin	Brine			Skin	Brine
days		%	*no./cm*	*no./ml*		%	*no./cm*	*no./ml*
0	4.0	0.3	1.1×10^5	1.3×10^4	7.1	0.3	1.1×10^5	1.3×10^4
3	5.5	0.6	7.7×10^4	9.3×10^4	6.8	0.6	--	8.1×10^4
9	--	1.3	1.9×10^5	1.4×10^5	--	1.2	2.4×10^6	2.6×10^6
11	5.5	--	3.2×10^4	2.1×10^5	6.8	--	1.0×10^6	5.0×10^6
18	5.5	1.3	3.8×10^4	2.0×10^4	6.8	1.4	3.3×10^6	3.5×10^7

except for a small amount of suspended protein, remained clear and colorless.

The number of bacteria on the skin of the salmon held in the untreated brine increased more than 20 fold. After the salmon had been in storage for 9 days, the number of bacteria on the skin increased from its original value of 1.1×10^5 to 2.4×10^6/cm^2. At 11 days of storage, the salmon were judged, on the basis of odor, to be spoiled.

Swab tests on the skin of the salmon held in the brine treated with CO_2 showed that essentially no growth of bacteria occurred during the 17 days of storage.

Chemical Measurements

pH.—The pH of the brine was measured as was described earlier. The pH of the flesh was not measured. Table 3 shows the pH values for the brines.

Except for the initial value of 7.1, the untreated brine had a pH of 6.8 throughout the experiment. As yet, we do not know if the difference in pH of the brines used for holding rockfish and salmon is related to a difference in the spoilage patterns of the two species.

The pH of the brine treated with CO_2 remained in a stable acid condition throughout the experiment.

CO_2 concentration.—No analyses were made for CO_2 in the flesh or in the brine. However CO_2 was continuously metered into the experimental brine at the same rate as that in the experiment with rockfish.

Salt concentration.—For greater accuracy than is possible with the simple rapid method of analysis described earlier, the concentration of NaCl in the flesh was measured by the Volhard method (Horwitz, 1960). The sample analyzed was taken from both fillets of a single fish. The fillets were mechanically comminuted and thoroughly mixed before the sample was taken, and the analyses were made in duplicate.

As was true with the rockfish, treating the brine with CO_2 had no effect on the rate of salt uptake. Salmon held in both brines showed progressive and similar increases in concentration of salt to a maximum of 1.3% to 1.4% in the flesh at 9 days.

SUBJECTIVE MEASUREMENTS

Raw Salmon

At each sampling, the salmon were examined in the same manner as had been the rockfish.

At the beginning of the experiment, the untreated salmon had a bright appearance and a thick covering of colorless slime. After 4 to 5 days, however, they had lost their natural brightness. They remained slimy, but the slime had begun to turn yellow. By the 11th day, the salmon looked blanched and smelled spoiled. At this time, the brine was dark brown and had an intense odor of spoilage.

The salmon held in the brine treated with CO_2 retained most of their natural color during the experiment. By the end of the first week of storage, however, only a trace of slime remained on their skins. On the 18th day, when the experiment was terminated, the salmon still had a good appearance and were free of off odors. The brine was almost colorless and almost odorless.

Cooked Salmon

The taste-test scores (Table 4) show that the samples from both storage environments were equally acceptable through the first 7 days of storage.

TABLE 4.—Sensory evaluations on the flesh of chum salmon held in CO_2-treated refrigerated brine and in untreated refrigerated brine.

Time in storage	Overall sensory score[1]		Comments	
	Brine and CO_2	Brine (control)	Brine and CO_2	Brine (control)
days				
0	9.0	9.0	--	--
3	8.0	8.0	--	Slight salty taste
7	8.0	8.0	--	--
11	7.0	Spoiled	Unobjectionable salty taste; firm texture	Odor of uncooked flesh putrid
18	6.0		Color, odor, and texture good; objectionable salty taste	--

[1] A score of 10 denotes a product of highest quality; one of 5 denotes a product of borderline quality.

By the 11th day, however, the taste panel rated those held in the untreated brine as being unacceptable.

In contrast, the salmon held 18 days in the treated brine were acceptable. The panel judged that these salmon had good texture and color but that they had only fair flavor. The deterioration in the flavor may have been due in part to the presence of absorbed salt (a salt concentration of about 1.0% is generally considered to be optimum) but was due mostly to chemical changes that occurred in the flesh during storage.

SUMMARY AND CONCLUSIONS

The purpose of the work reported here was to determine the effect that holding rockfish or chum salmon in refrigerated brine treated with CO_2 would have on their storage life and quality.

Storing rockfish in brine treated with CO_2 increased their storage life by at least 1 week. The CO_2 inhibited bacterial growth and retarded the rate at which the rockfish decreased in quality.

Storing chum salmon in brine treated with CO_2 gave similar results.

This study indicates that the addition of CO_2 to refrigerated brine considerably improves the preservation properties of this medium with respect to bacterial spoilage. The absorption, however, of water, uptake of salt, loss of soluble protein, and the as-yet-undetermined subsequent refrigerated shelf life of the landed product are problems that remain to be solved. At this time, we therefore cannot recommend that rockfish and chum salmon be held in modified refrigerated brine beyond presently accepted storage periods—that is, 8 to 10 days for either species.

Although we do not at present recommend extending the holding times, the reader may wish to keep in mind that the quality of a landed product held in refrigerated brine is significantly improved by the addition of CO_2.

Future modified brine studies will be directed at solving the above mentioned problems and the problems concerned with accelerated corrosion.

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DDT RESIDUES IN SEAWATER AND PARTICULATE MATTER IN THE CALIFORNIA CURRENT SYSTEM

JAMES L. COX

ABSTRACT

Continuous samples of seawater and organic particulate material collected along linear transects in the California current system were analyzed for DDT residues. DDT residue concentrations in whole seawater, as determined by continuous-flow, liquid-liquid extraction, ranged from 2.3×10^{-12} g/ml off Oregon and Washington, to 5.6×10^{-12} g/ml off southern California. Geographical patterns in these concentration values are discussed in relation to mechanisms of land-sea DDT residue transfer. DDT residue concentrations in particulate material collected by continuous-flow centrifugation and filtration of the centrifugal pellet onto GFC-glass-fiber filters, ranged from 1.2 to 5.7×10^{-6} g/g carbon (with one exception). These values were related to the density of the standing crops. DDT residues in this particulate fraction accounted for less than 10% of the DDT residues in the whole seawater samples. Residues which are fixed to particles of less than 1-2 μ in diameter may account for the balance of the DDT residues in the whole water samples. Experimental results are described which implicate adsorption as the uptake mechanism for algal cells; these experiments also support the idea that <1-2 μ diameter particles carry most of the DDT residues in whole seawater.

DDT and its metabolites have dispersed into the ocean and are found in high concentrations in the predators of oceanic food chains. Theoretical considerations predict a net transfer of extant DDT residues to the oceans, via atmospheric and river currents (Smith, 1970). In view of the well-known chemical stability of the principal constituents of the DDT complex, *p,p'*-DDT, DDD, and especially DDE, it is not surprising that levels of DDT residues in marine plankton samples have risen during the past decade (Cox, 1970a). No published data are available, however, on concentrations of DDT residues in seawater and in oceanic particulate matter. Chlorinated pesticides have been found in concentrations up to 13×10^{-9} g/ml in surface slicks in Biscayne Bay, Fla., and at concentrations of about $^{-12}$ g/ml in the surrounding waters (Seba and Corcoran, 1969). Measurements of DDT concentration in the open ocean are needed to construct a systematic account of DDT residue transport to the pelagic environment of the ocean, and to estimate the ultimate transport of DDT residues to the sediments.

METHODS AND MATERIALS

Samples of water and particulate material were collected during cruises of the RV *Proteus* in May 1970 from Monterey Bay, Calif., to San Diego, Calif., passing outside the islands off the southern California coast and returning closer to shore through the Santa Barbara Channel. A second cruise was made in September 1970 from Vancouver, British Columbia, to just off the mouth of San Francisco Bay, Calif. Figures 1 and 2 show the cruise tracks and the station enumeration for these cruises.

Sampling was continuous and was done while the ship was underway. Water was obtained from the shipboard seawater system (PVC and Teflon) which pumped water from about 1-2 m below the surface. The stream was first filtered through a 0.176-mm mesh net to remove larger zooplankton from the sampled water. The stream was then split; part of the water was directed into a peristaltic pump which metered the flow of particle-bearing water into a continuous-flow, internal recycle and recovery, liquid-liquid extracter of the type described by Kahn and Wayman (1964). Flow rates through the liquid-liquid extracter averaged 480 ml/hr.

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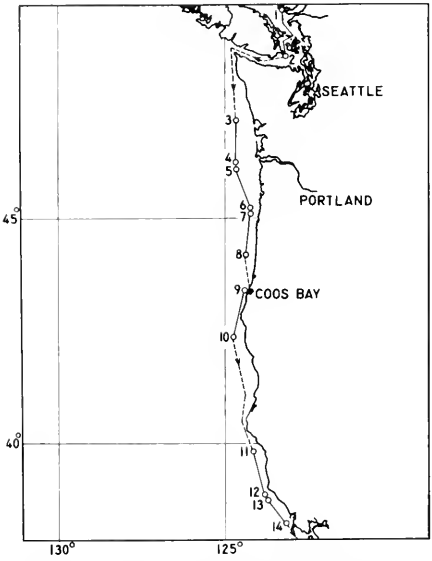

FIGURE 1.—Chart of the transects from Vancouver, British Columbia, to San Francisco Bay, Calif. See Tables 1 and 3 for station data.

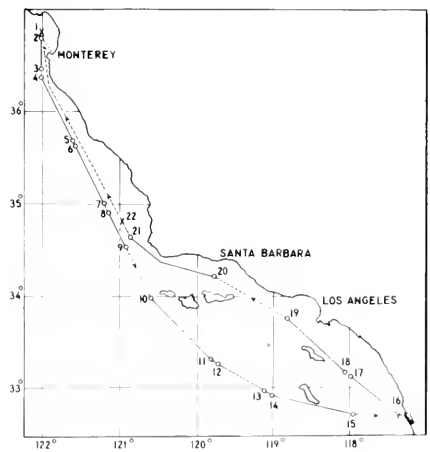

FIGURE 2.—Chart of the transects from Monterey Bay, Calif., to the southern California area. See Tables 2 and 4 for station data.

Since only one extracter was used for water extraction, the possibility existed for incomplete recovery of the DDT residues in the water passing through the device. Repeated tests of the extraction efficiency using a large carboy of oceanic seawater labelled with low levels of ^{14}C-DDT (ca. 5×10^{-12} g/ml) gave an extraction efficiency of 83% (±5%) at the flow rate settings which were used with apparatus at sea. A Variac setting of 70 was used, which produced an internal recycle rate of 900 ml/hr. The magnetic stirrer rate, which affects the degree of fracture of the solvent droplets, was kept constant.

At the end of a particular run, the contents of the centrifuge tubes were transferred to combusted 4.25 cm Whatman GFC filter papers[2] and stored in glass petri dishes at —15° C until analysis. The samples from one tube were analyzed for particulate carbon by the wet combustion method of Strickland and Parsons (1968). The samples from the other tube were analyzed for DDT residues according to previously described methods (Cox, 1970a).

At the end of a water extraction run, the flask containing about 100 ml of hexane, the water extract, was removed and stored until processing. The extract was condensed to 100 μliters after dehydration by passage through an Na_2SO_4 column. The Na_2SO_4 was specially rinsed with solvent and combusted at 350° C to remove interfering impurities normally present in the reagent salt (Lamar, Goerlitz, and Law, 1966). The condensed extract was spotted on an alumina chromatoplate, which was developed in 5% benzene in hexane, so that the solvent front moved 10 cm from the origin. Centimeter wide zones were stripped from the chromatoplates which corresponded to zones expected to contain *p,p'*-DDT, DDD, and DDE according to spots on parallel chromatograms with pure standards. ^{14}C-DDT and ^{14}C-DDE were also used to determine R_f zones. These zones

[2] The use of trade names is merely to facilitate descriptions; no endorsement is implied.

were eluted with a small amount of 20% benzene in hexane into test tubes. The eluates were analyzed individually by the same gas chromatographic techniques used for the particulate samples.

All glassware was combusted at 350° C overnight to remove interfering contaminants. All solvents were nannograde or pesticide quality. A hexane blank was run through the same procedure to detect systematic errors from any of the steps after the initial extraction. No correction was found to be necessary.

RESULTS AND DISCUSSION

WHOLE SEAWATER EXTRACTS

Comparisons of DDT residue concentrations in the particulate samples obtained by the centrifugation/filtration method described above (hereafter referred to as the particulate material) are meaningless when they purport to describe geographical differences since these concentrations change according to the density of the standing crop of the particulate material (Cox, 1970a). Comparisons of the concentrations of DDT residues in whole seawater (Tables 1 and 2) reveal some significant geographical differences. Water in the southern California region appears to have a higher DDT residue concentration. Water off Oregon and Washington has lower concentrations, and there is no evidence of high DDT residue levels adjacent to the mouth of the Columbia River. The relative uniformity of the DDT residue concentrations for this northern cruise (Table 1) suggests a diffuse source of the residues, possibly from atmospheric fallout. Direct measurements of the DDT content of dust in the atmosphere over the Atlantic Ocean (Risebrough, Hugget, Griffin, and Goldberg, 1968) and measurements of DDT residues in rainwater (Tarrant and Tatton, 1968; Yates, Holswade, and Higer, 1970) implicate aerial transport as an important mechanism of land-sea DDT residue transfer. Published calculations based on annual rainfall statistics and probable DDT residue concentrations in rainwater predict the concentration of DDT residues in the surface mixed layer of the oceans to be 5×10^{-12} g/ml (Smith, 1970). This estimate is within a factor range of 0.5 to 1.1 of the results presented in Tables 1 and 2.

Atmospheric fallout may be important in areas remote from river systems draining agricultural areas or in areas remote from waste dumping of highly populated areas. Sewage outfalls near large centers of population, such as the southern California area, contribute a large share of the DDT residue input to the ocean. When the outfall is below the pycnocline, the DDT residues in the effluent may settle with the particles comprising the solid component of the sewage and thus enter the benthic environment. This may account for the high DDT levels found in the livers of bottom dwelling fish in the southern California region, as compared to pelagic species (figures released by the California Department of Fish and Game in 1970). Sedimentation of organic particulate material from the surface layers represent an additional input to the benthos.

Input of DDT residues to the mixed layer is represented by the following sources: (1) sewage input by vertical transport of material from below the pycnocline or by direct input from shallower outfalls, (2) input from terrestrial runoff water which bears fallout particles, and (3) direct input from fallout of particles over the water. The relative importance of these

TABLE 1.—DDT residue concentrations in seawater obtained by liquid-liquid whole water extracts from transects shown in Figure 1.

Stations	Total volume extracted (liters)	DDT concentrations in water-ports per 10^{12}
1-2	2.6	2.3
3-8	4.1	2.7
9-10	2.8	2.3
11-14	4.3	2.3

TABLE 2.—DDT residue concentrations in seawater obtained by liquid-liquid whole water extracts from transects shown in Figure 2.

Stations	Total volume extracted (liters)	DDT concentrations in water-ports per 10^{12}
4-7	2.8	4.1
10-13	4.0	3.0
14-15	1.6	5.6
16-19	3.3	3.4

sources is not known, but it is quite likely that sources (1) and (2) account for the higher DDT residue concentration in the whole seawater samples taken off southern California.

PARTICULATE MATERIAL

Results of the analyses of the particulate material are shown in Tables 3 and 4. Transect 10-11 (Table 4) yielded an abnormally high value when compared to the other values for particulate material. During transect 10-11, visual observations were made of oil globules at the sea surface. The abnormally high value may have been caused by inclusion of a small globule of this material in the particulate material for transect 10-11, after entrainment in the seawater system of the vessel. This value has been deleted from further data presentations.

TABLE 3.—DDT residue concentrations in organic particulate material collected by continuous-flow centrifugation and collection of the centrifugal pellet on GFC-glass-fiber filter papers. Transects shown in Figure 1.

Tronsect stations	Total volume filtered (liters)	Wt of carbon in centrifugal pellet (g × 10−6)	DDT concentration μg DDT residues/g carbon (ppm)
1-2	48	4,500	1 4
3-4	48	2,980	2 2
5-6	29	2,550	1 8
7-8	17	490	5 7
9-10	39	2,680	2 1
11-12	24	1,780	2 3
13-14	28	600	8 3

TABLE 4.—DDT residue concentrations in organic particulate material collected by continuous-flow centrifugation and collection of the centrifugal pellet on GFC-glass-fiber filter papers. Transects shown in Figure 2.

Transect stations	Total volume filtered (liters)	Wt of carbon in centrifugal pellet (g × 10−6)	DDT concentration μg DDT residues/g carbon (ppm)
1	[1]	1725	27.0
2-3	44	4700	1 6
4-5	36	2130	2.4
6-7	37	2750	2 4
8-9	23	2140	2 5
10-11	36	3320	16 0
12-13	19	2330	1.5
14-15	33	2690	1 4
16-17	50	3280	1.6
18-19	60	3030	1.2
20-21	42	4010	1.3
22	[1]	3770	1.2

[1] These samples were obtained by using a net; see text for details.

On the May cruise to southern California (Figure 2), two phytoplankton ¼-m net tows (35-μ effective aperture) were taken at stations 1 and 22, and analyzed along with the particulate material samples. These tows consisted of 10 successive vertical hauls from 15 m to the surface at station 1 and one oblique haul from 10 m to the surface at station 22. The station 1 value is in approximate agreement with earlier published DDT residue concentrations for net phytoplankton samples (27 ppm per unit of carbon converts to 0.27 ppm wet weight; compare to values given by Cox, 1970a). This value is considerably higher than the values listed in Tables 3 and 4 for particulate material. At station 22, the ship was stopped for an investigation of a dense phytoplankton bloom, which consisted principally of *Rhizosolenia* spp. No measurements of chlorophyll were made, but the water was visibly discolored due to the high concentration of algal cells in parallel streaks at the surface. The concentration of DDT residues in net-tow material from this bloom was considerably lower than in the sample taken at station 1 (0.012 ppm wet weight compared to 0.27 ppm). This may be explained by the fact that the standing crop density was much higher at station 22 than at station 1.

The generally lower values in the particulate material compared to net-tow material (except in the case of station 22 as discussed above) could result from at least three causes: (1) loss of materials by cells bursting during the centrifugation (filtration as a cause of bursting of cells is well known, but cannot account for a difference in this case since the net-tow samples [Cox, 1970a and this report] were vacuum filtered through GFC papers as well), (2) inclusion of smaller particulate material having a lower intrinsic DDT residue concentration, or (3) exclusion from the centrifuge of larger zooplankters which would be trapped by the phytoplankton net.

Cause 1 represents one reasonable source of loss of DDT residues from the particulate material, if in fact they should have higher DDT residue concentrations than those reported herein. However, experiments with the same cen-

trifuge showed that at least 98% of the particulate chlorophyll *a* in the incurrent water is recoverable from the centrifugal pellet in whole particulate form (trappable on GFC filters). This indicates that breakage of cells must be minimal.

Cause 2 is also a possible explanation. Pfister, Dugan, and Frea (1969) pointed out that chlorinated hydrocarbons showed quantitative differences of distribution among particles greater than 0.15 μ which were separable by density gradient centrifugation. Although they found no recurrent patterns of distribution among the DDT metabolites they were able to detect, their results suggest large differences in the pesticide concentrations in the four different density classes of particles analyzed. The form in which their data are presented, however, does not allow any conclusions about lower or higher DDT residue concentrations in the material which was collected in the centrifuge, but not included in the net-tow material.

Odum, Woodwell, and Wurster (1969) found lower DDT residue concentrations associated with smaller detrital particles in a core taken from a sprayed marsh, but it is uncertain if these results may be applied to oceanic seston.

Cause 3 is a possible explanation on the basis of the mesh size of the zooplankton exclusion filter used in the centrifugation filtration procedure (0.176 mm) compared to the one used in the processing of the net-tow material both in this report and the earlier published data (0.33 mm).

EFFECT OF STANDING CROP DENSITY

The effect of standing crop density, alluded to above, was observed in the analyses of the particulate material. Standing crop densities were calculated for the transects using estimates of the volume of water filtered during the centrifuge running time and the carbon analyses of the centrifugal pellet. The values for DDT residue concentration are plotted vs. the standing crop density in Figure 3. The slope of the regression line fitted to the data points from both cruises is approximately —1, indicating that equal amounts of DDT residues were taken up by the algal materials within a given volume of water over the range of standing crop densities encountered. This is essentially the same conclusion mentioned earlier (Cox, 1970a).

PARTICULATE MATERIAL AS A PART OF WHOLE SEAWATER

Data points from the Vancouver to San Francisco cruise seem to fit the empirical linear relationship detailed in Figure 3 much more closely ($r = -0.99$) than the data points from the Monterey Bay to southern California cruise ($r = -0.54$). This variability is undoubtedly due to the greater variability of the DDT residue concentrations of the whole seawater from the southern California region, where most of the samples were taken. There would be no need to impute causal relationships between the whole seawater concentration and the concentration of DDT residues in the particulate material, if the particulate material represented a major portion

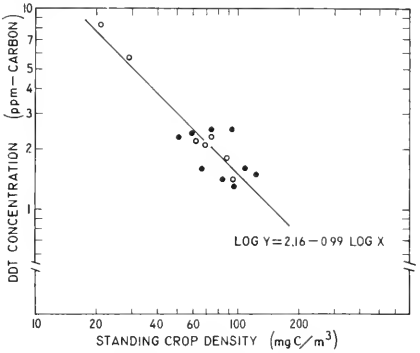

FIGURE 3.—DDT concentrations in the particulate samples as a function of particulate carbon standing crop density. Stations 1 and 22 (Table 4) are not included, since the density of the standing crops could not be computed because there were no measurements of the volume of water filtered in these net-tow samples. Also, for reasons outlined in the text, they may not be comparable to the samples collected by the centrifuge. Transect station 10-11 (Table 4) was omitted because of the possible interference of oil, as described in the text. The remaining 16 values from Tables 3 and 4 appear in this figure. Open circles refer to data from Table 3; solid circles refer to data from Table 4.

of the DDT residues in whole seawater. In fact, the particulate material accounted for less than 10% of the DDT residues in the corresponding whole water extracts (range: 1.8% to 9.9%). Unless the remaining amount of DDT residues (<90% of the total present) is in soluble form, it must be fixed to particles not collected in the centrifugation/filtration procedure. Typical natural distributions of particulate matter in seawater (Bader, 1970; Beardsley, Pak, and Carder, 1970) suggest that most of the particulate volume and almost all of the particulate surface area is accounted for by particles of less than 2 μ in diameter. Thus it is quite likely that the balance of the DDT residues in whole seawater are fixed to these smaller particles, in view of the hydrophobicity and affinity for interfaces characteristic of the different metabolites of DDT. The possibility also exists that it may occur as micelles or aggregates which cannot be taken up by the particulate matter.

EXPERIMENTAL EVIDENCE

Two experiments were performed to examine the distribution of DDT residues between seawater and phytoplankton. In both experiments, ^{14}C-DDT in a 1-ml ethanol carrier was added to GFC filtered oceanic seawater in a 4-liter glass carboy which was stirred by a magnetic stirrer. Repeated subsamples of 25 ml each were taken from the system until successive samples gave a constant ^{14}C activity. All counts were made on a Nuclear-Chicago Unilux II scintillation counter.

Aliquots of a dense suspension of *Dunaliella salina* culture were added to the carboy from a large separatory funnel with a 25-ml dispensing chamber, via a tube connected to the carboy. Sampled and added amounts were such that a constant volume was maintained. After addition of an aliquot of culture, one or two aliquots of 25 ml each were removed from a tap at the bottom of the carboy. This amount was vacuum filtered onto a GFC-glass-fiber filter paper, and counts of ^{14}C-DDT were made of the filter and of a petroleum ether extract of the filtrate. Cumulative ^{14}C activity in the filter and filtrate equalled amounts present in the 25 ml aliquots (both filter and filtrate) before addition of the algal suspension, when the net amounts of ^{14}C-DDT removed from the system by sampling were taken into account. A correction was made for adsorption or possible trapping of small particles of ^{14}C-DDT on the filter. The correction factor, expressed as percent of total activity per 25-ml aliquot which was on the filter before addition of the algal suspension, was constant in the five replicates taken just before the algal cells were added. This correction factor may have changed during the course of addition of the algal cells, but the techniques used did not allow a distinction between ^{14}C activity on the filter which adsorbed, associated with trapped small particles, or associated with the algal cells themselves. I believe that this change was small and did not materially affect the outcome of the experiments.

Figure 4 shows the results of the two experiments. In Experiment 1, the seawater used in the carboy was not altered; in Experiment 2, the seawater was specially prepared to increase the load of small (<1-2 μ) inorganic particles, to see what effect this might have on the uptake function. Nuchar-attaclay, a mixture of finely divided charcoal and clay particles (attapulgite), was added to 2 liters of GFC-filtered seawater. After shaking, the mixture was refiltered through a GFC filter. It is estimated that only a tiny fraction of the initially added Nuchar-attaclay (initially added amount was 0.1 g) actually got through the filter. The 2 liters of water produced in this way were mixed with another 2 liters of GFC-filtered seawater and put into the carboy. Two other conditions were different in Experiment 2. The culture of *Dunaliella salina* used was denser (note that the arrow in Figure 4 indicates that 750 μg C/liter is reached at a lower volume of culture added). The initial concentration of ^{14}C-DDT in Experiment 2 was approximately 15 ppt.

The first part of the uptake functions in Experiments 1 and 2 appeared to be linear, indicating that under the conditions prevailing at the beginning of each experiment, each *Dunaliella* cell took up a constant amount of the ^{14}C-

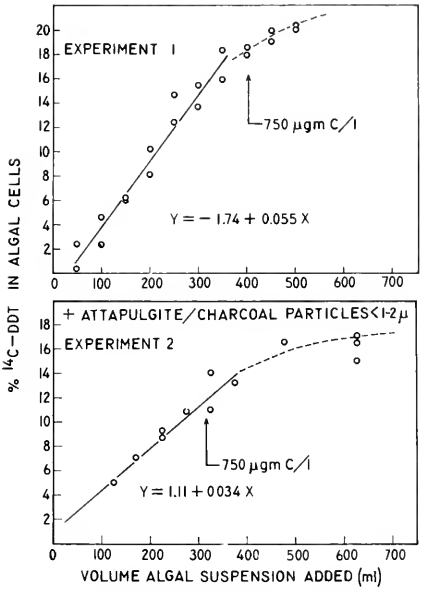

FIGURE 4.—Percentage of total ^{14}C-DDT in sample aliquots recovered on GFC filters, plotted as a function of total volume of *Dunaliella salina* culture added to a constant volume system. See text for detailed discussion.

DDT which was available. Both curves show inflection points after the density of cells increases beyond 750 μg C/l. The fact that the uptake per cell was constant over the linear range indicates that each cell has a saturation value for uptake of DDT, which is independent of the ambient concentration of DDT available. The curves presumably begin to level off when the ^{14}C-DDT which was available for uptake is mostly associated with the algal mass already added.

If algal cells exhibit a saturation value for uptake of DDT, then adsorption of DDT to the cell surface is a more likely explanation for DDT uptake than phase partitioning of DDT between seawater and the lipid component of the algal cell, as has been previously hypothesized (Cox, 1970b). Each *Dunaliella salina* cell probably had a total cell surface area of 240 μ^2 (Mullin, Sloan, and Eppley, 1966). The cells in Experiments 1 and 2 took up a mean of 5×10^{-5} picograms ^{14}C-DDT μ^2. This value may be near the asymptotic saturation value for *Dunaliella salina* for the experimental conditions described above. The validity of a saturation value of this kind needs to be tested with other phytoplankton species over a wide range of ambient DDT concentrations.

A quantitative solution to simultaneous Freundlich adsorption equations for the algal cells and the $<$1-2 μ particles could explain the uptake curves if the adsorption energy coefficient were known in each case. Studies such as those of Weber and Gould (1966) should therefore be applied to uptake of DDT residues by phytoplankton and smaller particles to elucidate the relationships discussed here.

No measurements were made of the concentration of the $<$1-2 μ particles in the untreated seawater of Experiment 1 or the treated seawater of Experiment 2. Thus the differences can only be explained qualitatively. The higher concentration of ^{14}C-DDT in Experiment 2 (30 ppt) was apparently reflected in the uptake of ^{14}C-DDT per unit of cell surface area; Experiment 2 yielded a value of about 6×10^{-5} picograms ^{14}C-DDT μ^2, which is higher than the mean for both experiments quoted above. The uptake of ^{14}C-DDT per unit of cell surface area in the case of Experiment 2 is probably closer to the asymptotic saturation value because of the higher concentration of ^{14}C-DDT in the medium. The main difference between the curves for Experiments 1 and 2 is the position of the inflection point. Experiment 2 shows an apparent inflection point which is lower than the apparent inflection point of Experiment 1, indicating a lowering of the available percentage of total ^{14}C-DDT in the system. The total small particle concentration of the system was not measured, so this apparent change must be regarded as a presumptive effect of the Nuchar-attaclay addition.

If a large percentage of the DDT added to aqueous systems is fixed to a particle fraction

less than 1-2 μ in diameter, then the experimental DDT uptake results that have been interpreted in terms of a partition coefficient or a concentration factor using the nominal concentration of DDT in the aqueous medium become relatively meaningless without knowledge of the fraction of the initial amount of DDT present which is fixed to these small particles and hence unavailable to the test organism. In the oceanic environment, it is quite likely that the amounts of "available" DDT residues are exceedingly small. Uptake of DDT by the seston is probably closely coupled with input pulses which would be largely determined by fallout conditions at the surface and seasonal runoff of DDT residues from land areas. "Available" DDT residues which may rise during these periods will be taken up by plankton. A complete picture of the processes involved in DDT transport to the pelagic environment has yet to be drawn and will require further experimental and analytical work.

ACKNOWLEDGMENTS

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EGG LOSS DURING INCUBATION FROM OFFSHORE NORTHERN LOBSTERS (DECAPODA: HOMARIDAE)

HERBERT C. PERKINS[1]

ABSTRACT

Egg loss during incubation from offshore northern lobsters, *Homarus americanus* Milne Edwards, was estimated by counting the eggs of 196 females. The lobsters were captured along the continental shelf off southern New England during October (eggs recently extruded), April, and June (eggs nearly ready to hatch). Egg loss during the period October to June averaged 36% for females of all sizes studied.

The exploitation of northern lobsters in the offshore canyons of the continental shelf is steadily increasing (Skud, 1969). Owing to this increased effort, the National Marine Fisheries Service (formerly the Bureau of Commercial Fisheries) has initiated a study of the biology and population dynamics of the stock. As accurate estimates of fecundity are useful for studying population dynamics, the present study was undertaken to determine the extent of egg loss from female *Homarus americanus* during embryonic development and the consequential magnitude of error in estimating fecundity if any loss occurs. Brunel (1962, 1963) has shown that female spider crabs, *Chionoecetes opilio*, may lose over half their eggs during the incubation period. The present paper shows the extent of egg loss from the northern lobster and the difference in fecundity estimates depending on the time during the development period when the eggs are counted.

METHODS AND MATERIALS

The lobsters were captured with otter trawls during research and commercial cruises at Hudson, Veatch, Oceanographer, Lydonia, and Corsair Canyons. These canyons are located along the edge of the continental shelf, south and east of New England. The 196 female lobsters used in this study were divided into 5-mm groups according to carapace length. The mean number of eggs and the range for each 5-mm group are recorded in Table 1. Carapace length was measured from the posterior edge of an eye socket to the distal edge of the carapace.

The females were frozen at sea, later thawed in the laboratory and their eggs removed from the pleopods by stripping with small forceps. The eggs were hardened in Formalin for 24 hr, then soaked in fresh water before being dried in an oven at 150° C. Drying time lasted 1 to 2 hr depending on the size of the egg mass. After drying, the individual egg masses were rubbed over a 1-mm screen to break up any clusters and eliminate non-egg material, then counted with an electronic counter (Boyar and Clifford, 1967). Test runs with the counter produced a maximum error of ± 2%. The counts given represent numbers of viable eggs only (nonviable eggs are rarely observed in masses of developing eggs).

RESULTS AND DISCUSSION

A curvilinear relationship was apparent when the number of eggs was plotted on the corresponding carapace length. The same result was noted by Saila, Flowers, and Hughes (1969). In order to employ covariance analyses in this study, the data were transformed to natural logarithms. The lines presented in Figure 1 were plotted from the antilogarithms of the values calculated from the linear regression equations (Table 2).

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TABLE 1.—Month of capture, range and mean number of eggs for each 5-mm carapace length group for the 196 offshore female lobsters.

Carapace length groups (mm)	OCTOBER			APRIL			JUNE		
	Number of lobsters	Number of eggs		Number of lobsters	Number of eggs		Number of lobsters	Number of eggs	
		Mean	Range		Mean	Range		Mean	Range
80-84	3	10,449	8,286-11,357	1	9,212	--	1	7,890	--
85-89	10	14,341	8,707-17,428	2	10,518	9,010-12,027	2	7,920	6,400- 9,440
90-94	23	16,317	11,501-21,348	4	13,476	9,578-18,400	5	8,950	6,410- 12,240
95-99	15	19,440	14,425-25,454	3	15,973	12,140-19,310	3	9,887	8,270- 10,700
100-104	15	20,463	13,831-26,832	8	19,355	12,853-23,270	6	14,900	8,470- 17,940
105-109	1	24,896	--	4	19,212	16,161-22,853	4	19,692	10,160- 30,190
110-114	3	30,452	27,321-32,309	11	23,789	17,639-28,245	0	--	--
115-119	0	--	--	7	27,001	18,703-33,160	1	30,602	--
120-124	2	44,334	42,059-46,610	1	26,628	--	3	27,743	20,671- 31,789
125-129	2	51,184	50,008-52,361	5	35,757	18,821-40,159	1	42,743	--
130-134	0	--	--	5	32,796	25,180-41,636	4	40,728	33,238- 49,820
135-139	1	55,240	--	2	31,045	26,850-35,240	4	40,092	22,600- 52,956
140-144	0	--	--	2	51,002	48,526-53,478	4	45,506	32,770- 54,820
145-149	0	--	--	1	45,928	--	2	68,510	67,660- 69,360
150-154	0	--	--	0	--	--	5	52,457	44,463- 69,095
155-159	0	--	--	1	78,422	--	3	51,978	37,400- 66,551
160-164	0	--	--	1	66,210	--	3	64,673	56,302- 73,207
165-169	0	--	--	0	--	--	2	63,175	62,573- 63,777
170-174	0	--	--	1	70,178	--	3	71,831	60,866- 87,303
175-179	0	--	--	0	--	--	1	81,164	--
180-184	0	--	--	0	--	--	3	73,770	56,372-104,541
185-189	0	--	--	0	--	--	0	--	--
190-194	0	--	--	0	--	--	1	78,844	--
195-199	0	--	--	0	--	--	1	90,400	--

TABLE 2.—Raw sums, sums of squares, sums of cross products, and regression equations derived from transformed data on carapace lengths and numbers of eggs from 196 berried female lobsters captured during October, April, and June. ($X = \log_e$ carapace length, $Y = \log_e$ number of eggs.)

Month	N	ΣX	ΣX^2	ΣY	ΣY^2	ΣXY	Regression equation ($Y = a+bX$)
October	75	342.8625	1568.1306	737.7546	7267.0013	3375.0329	$\hat{Y} = -5.0202+3.2499(X)$
April	59	279.4272	1324.7648	595.6230	6026.4485	2824.7918	$\hat{Y} = -3.2280+2.8132(X)$
June	62	301.6997	1471.6059	640.9857	6666.0539	3130.1578	$\hat{Y} = -5.0231+3.1569(X)$

The regression lines were all significantly different ($P < 0.01$) in level (Y intercept) from each other. No significant difference was found in the slope of any of the lines, indicating egg loss is consistent between extrusion and hatching from females of all sizes. The average total loss from extrusion to hatching throughout the size range of the females studied was 36%, and the average loss during the last few months (April through June) was 13%.

The eggs obtained in October were all in prenauplíar condition (Templeman, 1940) and were judged to be no more than 4 weeks in age. Those eggs obtained in June were all within a month of hatching. Twenty-four berried females, caught in June, were kept in laboratory tanks where all egg hatching was completed by the middle of July. Eggs taken in April showed less development than those taken in June, and 12 females kept in the laboratory tanks from the April samples completed hatching by the first of July. Water temperatures in the laboratory during April and May were slightly higher than would be expected in the offshore waters for the same period.

The offshore waters where the lobsters are found undergo no great seasonal temperature fluctuations (Colton et al., 1968) typical of the coastal waters of New England. I have examined over 500 berried females from the offshore area and conclude that extrusion of eggs occurs in September and October, and that hatching occurs the following June or July. I have found no deviation from this pattern; however, slight

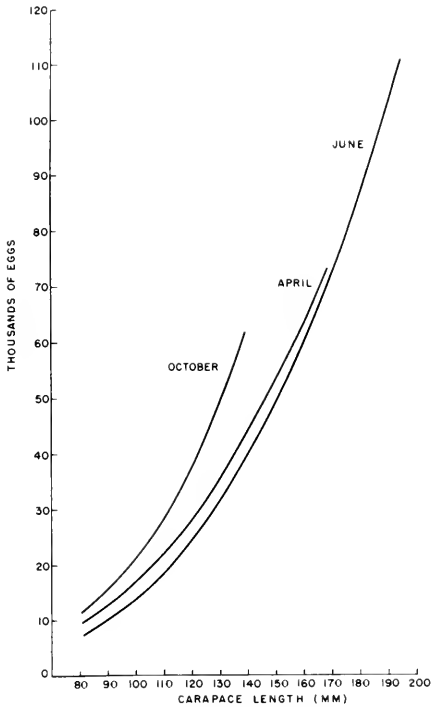

FIGURE 1.—Regression lines of egg counts versus carapace lengths for October, April, and June.

variation in the extent of development of eggs from one year to the next during the same month has been noted, and from canyon to canyon during the same year. These variations among years and areas are attributed to small differences in water temperature, while the consistent year to year reproductive periods are attributed to the relatively constant thermal environment.

While counts obtained from ovarian or newly extruded eggs testify to the reproductive potential of a female, counting eggs that are close to hatching give more reliable estimates of a female's potential contribution of larvae to the population.

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ERRATA

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ROTHSCHILD, BRIAN J., AND JAMES W. BALSIGER, "A linear-programming solution to salmon management," p. 117-140.

1) Page 120, left hand column, first line below equation (4) has the inequality in the wrong direction and should have:

"The constraint is redundant if $K' \geqslant \sum_{j=1}^{N} K_j$."

2) Page 120, right hand column, fourth line below equation (8) should have same script "$\prime\prime$" as "$\prime\prime$" in equation 8.

3) Page 120, right hand column, equation (9) has the inequality in the wrong direction and should be:

$$\Sigma W_{ij} \geqslant \frac{E \times H}{F}$$

4) Page 137, right hand column, line 40 should read:
... a series of years "maximizing" ...

SECKEL, GUNTER R., AND MARIAN Y. Y. YONG, "Harmonic functions for sea-surface temperatures and salinities, Koko Head, Oahu, 1956-69, and sea-surface temperatures, Christmas Island, 1954-69," p. 181-214.

1) The senior author's name is misspelled. It should be:
"Gunter R. Seckel," rather than "Gunther R. Seckel."

INTERNAL DEFENSES OF CRUSTACEA: A REVIEW[1]

CARL J. SINDERMANN[2]

ABSTRACT

Studies of the internal defenses of Crustacea have a discontinuous history, which began in the late 1800's. Elaborate early studies of phagocytes and humoral factors in the hemolymph have been extended with renewed vigor during the past decade. As is true for other invertebrates studied, phagocytosis of foreign particles by fixed and mobile cells in the crustaceans is augmented by naturally occurring bactericidins, lysins, and agglutinins. A few instances of experimental enhancement of titers of such humoral factors by previous exposure to foreign protein have been reported. Specificity of natural and experimentally enhanced humoral factors is much lower than that of vertebrate immunoglobulins, but probably such factors act synergistically with cellular protective mechanisms, as they do in the vertebrates.

Phagocytosis by fixed and mobile cells in gills, pericardial sinus, and sinuses at the bases of appendages seems to be a principal defense perimeter in many crustaceans. Efficacy of phagocytes in destroying invading microorganisms varies, depending on the species of the microorganism, as well as host physiology and environmental factors. Phagocytic activity is enhanced by hemolymph factors, which, in addition to immobilizing and agglutinating the invading organisms, also sensitize them to phagocytosis.

Hemolymph factors, most of which seem to be of cellular origin, may also have bactericidal or lytic activity, leading to extracellular destruction of microbial invaders. A few recent studies indicate that effects of hemolymph factors may be enhanced experimentally by injection of killed or living microorganisms.

The number of known microbial diseases in crustaceans is greater than that known in most other invertebrate groups, with the possible exception of the Mollusca and Insecta. The number and depth of studies concerned with internal defense mechanisms of Crustacea are similarly greater than those of most other invertebrate groups—again with the possible exception of the mollusks and insects. One microbial pathogen of Crustacea that has received adequate attention is *Gaffkya homari*—a gram-positive coccus which causes a fatal septicemia in lobsters and is capable of infecting other decapods. An elaborate series of studies in several laboratories has elucidated many details of the host-parasite relationship and has also provided extensive information about the internal defenses of a number of the larger Crustacea. *Gaffkya* constitutes a test microorganism of choice for future studies of disease processes in crustaceans.

Thus the available information about cellular and humoral defenses of Crustacea against invasion by foreign protein constitutes a significant part of what we know about such processes in the invertebrates. Phagocytosis, augmented by humoral factors with low specificity, seems to be the fundamental means of internal protection in the crustaceans and in other groups of invertebrates as well.

Investigations of the internal defense mechanisms of invertebrates against disease have progressed with renewed vigor in the past decade. Literature has accumulated to the point where condensation and summarization of information about certain invertebrate groups, such as the Crustacea, seem justified.

The large number of published reports on internal defenses of Crustacea can provide a good indication of the extent of our knowledge of immunity in marine invertebrates. Beginning with the pioneering work on phagocytosis and the entire process of inflammation by Metchnikoff (1884, 1893, and 1905), and on humoral factors described in the very extensive (but sometimes poorly documented, in terms of procedures and data) work of Cantacuzène (1912-34), the Crustacea have often been animals of choice in studies of internal defense mechanisms. A significant

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body of literature has accumulated, with conspicuous bulges in the early years (1884-1930) and in the past decade (1960-70), but with a very narrow waist during the period 1930-60.

Studies of humoral defenses of the larger Crustacea during the past 5 years (1965-70) are curiously reminiscent of work reported by Cantacuzène and his associates during the period 1912 to 1934, with the very important difference that most of the modern work includes elements that were largely missing from earlier reports, such as details of procedures, supporting data, adequate controls, and attempts at quantitation of results.

Because so much of the literature produced before 1940 lacks adequate quantitation and fails to provide details of techniques used, it is often difficult to relate results to those of more recent studies. Bang (1967b) has deliberately addressed himself to a repetition of earlier studies but has used modern methods in an attempt to improve the relationship. Other recent reports, even though based on species other than those used in earlier studies, constitute reexaminations of the concepts and general findings of the early investigators.

It is difficult to determine why the early work on immune responses in invertebrates so effectively begun by Metchnikoff, Cantacuzène, Cuénot, Bruntz, and others during the late 19th century and the early 20th century seemed to lose impetus and then virtually cease until recently. It is apparent from the literature, though, that research on invertebrate defenses, initiated so auspiciously, receded for a number of decades to the backwaters and eddies of the mainstream of advances in immunology, which was concentrated on the homoiothermic vertebrates. This may be explained in part by a natural and necessary concentration of research interest on human and mammalian immune responses (most immunologists were—and still are—generally associated with medical schools and hospitals). Part of the explanation also may be that earlier work failed to disclose any defense mechanisms in invertebrates that seemed fundamentally or conceptually different from those that were being elucidated for the vertebrates. More importantly, the explanation may have been that much of the earlier work failed to indicate any immunologic responsiveness in the invertebrates tested. In spite of occasional successes, the "inability of invertebrates to respond to the introduction of antigen by formation of antibodies" became a sort of dogma among many of the early biologists, as was pointed out by Cantacuzène (1923b). Failures to find responses may have been due partly to choice of inoculum with negligible antigenicity in the experimental invertebrate. Two factors may have caused the recent resurgence of interest among biologists in comparative immunology (which is gradually beginning to include the lower vertebrates and the invertebrates): (1) an evident need to reexamine the conceptual and evolutionary basis of immune responses and (2) an interest in understanding the internal defenses of invertebrates, which allow them to survive in a microbe-rich environment even though they lack the specific antibody response characteristic of most vertebrates.

The subject matter of the present review is one that has been treated previously (Cantacuzène, 1923b; Huff, 1940; Baer, 1944; Bang 1967b; Levin, 1967; Tripp, 1969; Rabin, 1970b; Bang, 1970). Many of those papers, however, were broad considerations of the invertebrates as a whole, and discussions of internal defenses of Crustacea were often more or less incidental. It is interesting—as Good and Papermaster (1964) pointed out—that no review of invertebrate immunity has emphasized induced responses. A recent, and excellent, 2-volume text on the physiology of Crustacea (Waterman, 1960) does not include a detailed consideration of the very important subject of internal defenses, except for reference to hemocytes and phagocytosis (Maynard, 1960; Parry, 1960), and a consideration of hemolymph coagulation (Florkin, 1960).

The general plan for this review is to discuss some of the early literature, after a brief preliminary statement about concepts and terminology, and a summary of known diseases of Crustacea. More recent studies will then be considered by categories of cellular and humoral systems: phagocytic, bactericidal, lytic, agglutinating, precipitating, phage clearing, and anti-

toxic. A final section will attempt a detailed review of the demonstrated systems of internal defenses of lobsters and other crustaceans against the microbial pathogen *Gaffkya homari* —which is one of the best examples of a test system for invertebrates for which existing information is adequate.

CONCEPTS AND TERMINOLOGY

Before proceeding with an examination of internal defenses of crustaceans, a brief review of some of the terminology may be relevant. "Resistance" and "susceptibility" have often been used interchangeably and reciprocally, but as Schneider (1951) and Stauber (1961) pointed out, insusceptibility and resistance should probably be considered as separate biological phenomena. Insusceptibility refers to those existing external or internal features of an animal —morphological and physiological—which deny access to a potential pathogen, or prevent its successful establishment and survival. Resistance, on the other hand, has been defined by Read (1958) as "those changes in the physiological state of the host which represent a *response* to present or previous contact with the parasite or with a similar chemical entity."

Resistance (and its equivalent—"immunity"), defined as host response, can then be considered as "innate" (natural) or "acquired" (induced). Innate resistance includes responses to primary contact with a pathogen, and acquired resistance includes responses that develop after primary contact. The distinction between the two types of response is not, however, always as definitive as might be preferred. Acquired resistance is often characterized by enhanced responsiveness to subsequent contact with a pathogen.

Resistance, whether innate or acquired, may be cellular or humoral. The cellular defense mechanisms are based largely on activities of leucocytes (hemocytes of invertebrates) and include: *phagocytosis*—engulfment and often digestion of foreign particles, *leucocytosis* (*hemocytosis*), and *leucocytic* (*hemocytic*) *infiltration*—the mobilization of hemocytes in the blood stream and their migration to invaded or injured tissues; *coagulation*—the formation of cellular or extracellular clots to close gaps in the circulatory system and to immobilize microorganisms; and *encapsulation*—the surrounding of large masses of invading material by phagocytes and fibrocytes. Humoral defense mechanisms, which probably depend on cellular secretions or cell disruption, and act synergistically with cellular defenses, include agglutinating, lytic, precipitating, and bactericidal systems, and other activities (Figure 1).

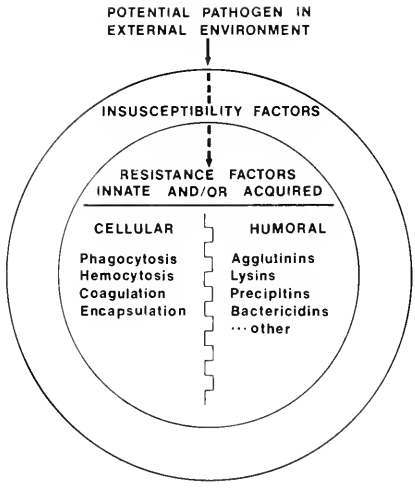

FIGURE 1.—Mechanisms of internal defense.

Antibodies (in the vertebrate sense of specific immunoglobulins) have not been demonstrated in invertebrates, although less specific antibody-like activity is common. Since antibodies have not been demonstrated, it is probably technically incorrect to use the term "antigen" with invertebrates. The semantics involved will be considered in the discussion section, but for convenience the term will be used in this paper. Furthermore, it must be made clear that when lysins, precipitins, agglutinins, etc. of invertebrates are discussed, no attempt is made to

homologize them with vertebrate factors. The terms merely indicate the type of activity produced.

DISEASES OF CRUSTACEA

An impressive array of diseases afflicting the Crustacea has been described (summarized in Sindermann, 1970, and Bang, 1970). A number of these diseases are of microbial etiology, and Koch's postulates have been satisfied for several of them. Some of the published reports concerning crustacean diseases include information about host defenses against infection, others do not. The literature also contains information about a variety of experimentally induced infections in Crustacea, many of them produced by microorganisms not known as pathogens in natural populations. Such experimental studies have been particularly useful in elucidating possible internal defense mechanisms—augmenting studies with known pathogens. The following summary is just that and is not intended as a detailed treatment of crustacean diseases. Some general background information on known diseases seems important, however, to any consideration of internal defense mechanisms.

The only virus disease of invertebrates reported in the scientific literature is one that occurs in crabs, *Portunus depurator*, on the French Mediterranean coast (Vago, 1966). Disease signs mentioned in his very brief paper included progressive darkening of the exoskeleton, paralysis, and death.

Bacterial infections of various Crustacea have been described, beginning with a disease of beach hoppers on the French coast caused by luminescent bacteria (Giard and Billett, 1889). Experimental infections were obtained by injecting cultured microorganisms, and some of the crustacean species tested exhibited varying degrees of resistance to experimental infection. Another luminescent disease was reported in sand fleas (*Talorchestia longicornis* and *Orchestia platinus*) from Woods Hole, Mass., by Inman (1927). Luminiscent bacilli were cultured, and experimental infections obtained. Luminiscent bacteria were also isolated from the digestive tracts of nonluminous sand fleas. A bacterial disease of *Gammarus marinus* was reported from England by Tait (1917), in which signs of disease included change in color of the infected amphipods from brown to opaque yellowish-white, reduction in numbers of blood cells, and absence of coagulation of hemolymph. Among the larger decapod Crustacea, a severe bacterial disease of lobsters caused by gram-positive cocci, *Gaffkya homari*, was recognized in 1947 (Snieszko and Taylor, 1947) and has been the subject of intensive studies since then (to be considered in detail later in this paper). Experimental infections and resultant mortalities of blue crabs, *Callinectes sapidus*, from Chesapeake Bay were reported by Krantz, Colwell, and Lovelace (1969) with *Vibrio parahemolyticus*. The microorganism has been isolated from mollusks, fishes, and sediments in various parts of the world and is known as a cause of human gastroenteritis in the Orient. Additionally, several examples of "shell disease"—erosion of the exoskeleton by chitin-destroying bacteria—are known in lobsters, crabs, and shrimps (Hess, 1937; Rosen, 1967, 1970; Anderson and Conroy, 1968).

Fungus diseases of Crustacea are surprisingly numerous in reports dating back to Metchnikoff (1884), who described fatal infections of *Daphnia* caused by *Monospora bicuspidata* and who first emphasized the crucial role of phagocytosis in determining the outcome of infection. A yeast infection in sand hoppers, *Talitrus*, from the coast of France, was reported by Herrmann and Canu (1891). Experimental infections from exposure to cultured microorganisms were fatal to *Talitrus* in 20 to 25 days. Phagocytosis was marked in such infections, and the hemolymph became milky in advanced cases. Crabs (*Carcinus maenas*), prawns (*Palaemonetes varians*), and crayfish (*Astacus fluviatulis*) were not susceptible to the experimental infections. Pixell-Goodrich (1928) described another yeast infection which was epizootic in *Gammarus* from a stream in England. The pathogen *Cryptococcus gammari* reproduced in the hemolymph and rendered it milky in color, coagulation was retarded, and heavily infected individuals died. Phagocytosis was active and sometimes successful in overcoming infections. Hypertrophy of fixed phagocytic cells was common.

One of the most severe, widespread, and long-continuing epizootics known in invertebrates has affected and still affects European crayfish. It is caused by the fungus *Aphanomyces astaci* (although other microorganisms have been variously associated with mortalities). Known as "Krebspest," the disease swept through crayfish populations of Europe beginning about the turn of the century (Schikora, 1906, 1926; Schäperclaus, 1935; Nybelin, 1935; Mannsfield, 1942; Unestam, 1965; Gordon, 1966). Apparently resistance differs among species—the American crayfishes, for example, seem less seriously affected by the pathogen in experimental studies.

Other fungus diseases of Crustacea include a systemic infection of pea crabs, *Pinnotheres*, from English sea mussels by *Leptolegnia marina* (Atkins, 1929, 1954a); a systemic disease of cultured prawns, *Palaemon serratus*, in England, caused by *Pythium* sp. (Anderson and Conroy, 1968); a gill infection of pandalid shrimp, *Dichelopandalus leptocerus*, from the western North Atlantic, caused by a chytrid-like microorganism (Uzmann and Haynes, 1969); and gill infections of lobsters, *Homarus vulgaris* and *Palinurus vulgaris*, in Italy, caused by *Ramularia branchialis* and *Didymaria palinuri*—both Fungi Imperfecti (Sordi, 1958).

Fungi also infect and destroy egg masses of Crustacea. Eggs of blue crabs, *Callinectes sapidus*, from Chesapeake Bay may be infected by *Lagenidium callinectes* (Couch, 1942; Newcombe and Rogers, 1947; Rogers-Talbert, 1948); and eggs of pea crabs are often infected by *Plectospira dubia* and *Pythium thalassium* (Atkins, 1954b, 1955).

Among the many protozoan diseases of Crustacea, those caused by microsporidans are probably the most destructive. *Nosema* sp. and *Plistophora cargoi* destroy body muscles of blue crabs (Sprague, 1965, 1966); *Nosema pulvis* and *Thelohania maenadis* infect muscles of green crabs, *Carcinus maenas* (Pérez, 1905a, 1905b, 1907). Other microsporidan infections of body muscles in Crustacea include those produced in *Gammarus* by *Theileria* sp. and *Nosema* sp. Necrotic muscle fibers containing microsporidan spores were destroyed by phagocytes (Pixell-Goodrich, 1928). Other pathological effects of microsporidans on gammarids have been recently reported by Bulnheim (1967) and Bulnheim and Vávra (1968). Crayfish muscles are attacked by Microsporida of the genera *Thelohania* and *Nosema* (Sprague, 1950b; Pixell-Goodrich, 1956; Sogandares-Bernal, 1962). Microsporida are also significant pathogens of shrimps. Sprague (1950a), Woodburn et al. (1957), Iversen and Manning (1959), Iversen and Van Meter (1964), and others have described infections of body muscles and gonads of shrimps from the Gulf of Mexico and European waters, caused by a number of representatives of the genera *Thelohania* and *Nosema*.

Other protozoan diseases of crustaceans include those caused by ciliates, an ameba, and gregarines. A ciliate, *Anophrys sarcophaga*, causes a fatal disease in shore crabs, *Carcinus maenas*, of Europe. The disease, and host responses to infection, will be considered in detail in a later section. Other parasitic ciliates occur in the hemolymph of Crustacea. *Paradinium* sp. and *Syndinium* sp. occur in calanoid copepods. *Syndinium* causes gonad destruction, while *Paradinium* colors the host a deep red (Gordon, 1966). *Hematodinium* sp. has also been reported by Gordon in *Carcinus*. A suctorian, *Ephelota gemmipara*, can seriously reduce production of lobster larvae (Dannevig, 1928, 1939). An ameboid parasite, *Paramoeba perniciosa*, causes a fatal disease (called "gray crab disease") in blue crabs from the Atlantic coast of the United States (Sprague and Beckett, 1966, 1968; Sprague, Beckett, and Sawyer, 1969; Sawyer, 1969). Hemolymph of infected crabs becomes cloudy and often incoagulable; in some individuals most of the cells in the hemolymph are amebae (Sawyer, Cox, and Higginbottom, 1970). A great number of gregarines occur in Crustacea of all kinds, but their pathogenicity seems slight, except for some destruction of the digestive epithelium resulting from heavy infections (Ball, 1938; Théodoridès, 1961, 1962; Tuzet and Ormières, 1961; Kruse, 1959a, 1959b).

Helminth diseases of crustaceans seem less abundant and less severe in their effects than those of microbial etiology. Trematode metacercariae encyst in muscles and hepatopancreas of

crabs, and larval cestodes, acanthocephalans, nematodes, and leeches occasionally have been reported from crabs, shrimps, and lobsters (Sindermann, 1970).

Crustaceans are frequently parasitized by other crustaceans—sometimes with serious effects on the host. Rhizocephalan barnacles are endoparasites of crabs, causing gonad degeneration and other morphological changes. Epicaridean isopods may produce similar changes in crabs and shrimps. Copepods sometimes parasitize crab eggs, as well as the gills of lobsters (Sindermann, 1970).

INTERNAL DEFENSE SYSTEMS

Studies of crustacean internal defenses published during the last decade have augmented earlier studies and have provided additional data to support generalizations and principles already enunciated, but none has yet provided the factual basis for new or different concepts. Because additional precision in terminology is now available, it is possible to consider the internal defense systems of Crustacea under the following headings: cellular (phagocytic), bactericidal, lytic, agglutinating, precipitating, phage clearance, antitoxic, and others. It should be obvious that these systems are not mutually exclusive and may often interact or even share components to protect the individual animal from invasion by potential pathogens. Largely for ease of description, the systems will be considered consecutively, even though many may act either in concert or simultaneously.

PHAGOCYTOSIS AND OTHER CELLULAR DEFENSES

The earliest study of phagocytosis in Crustacea concerned infections of *Daphnia* by the fungus *Monospora bicuspidata* (Metchnikoff, 1884). The fungus spores in the haemocoel were phagocytized and digested; the rapidity and vigor with which phagocytosis occurred determined the outcome of the infection. If some spores escaped phagocytosis, germinated, and formed conidia, the infection became generalized and the host died in a few days. If all the fungus spores were phagocytized and destroyed, the infection was arrested. Thus the speed and effectiveness of phagocytic action in some individuals, possibly mediated by humoral factors, determined survival. Absence of phagocytosis inevitably led to death.

Hemocytes of Crustacea were investigated by Cattaneo (1888b), Cuénot (1895, 1897, 1905), and Bruntz (1907). Cattaneo described the amebocytes of *Carcinus maenas;* Cuénot reported blood forming tissues—nodules of lymphoid cells in the blood sinuses—in decapods and described "phagocytic organs" in the hepatopancreas of decapods and amphipods; and Bruntz published an extensive paper on the hemocytes of many of the crustacean groups, distinguishing granular and hyaline hemocytes. Bruntz also described a "phagocytic organ" in gammarids; his 1907 paper reviewed an extensive series of his own studies (15 reports) published during the period 1903-1907 by the Societé Biologique de Paris. Other early studies of crustacean hemocytes include those of Hardy (1892), Tait (1918a, 1918b), and Tait and Gunn (1918).

Following the classical early studies of Metchnikoff, Cuénot, Bruntz, and others, which elucidated the critical role of phagocytes in the internal defenses against microorganisms, phagocytic cells have received greatest attention from vertebrate immunologists. General principles that have emerged from the more recent studies of phagocytosis in vertebrates undoubtedly apply as well to invertebrates. Among the papers that have contributed to understanding of phagocytosis are those of Wright and Douglas (1903), Wood, Smith, and Watson (1946), Wood (1953), Robineaux and Frederic (1955), Suter (1956), Rowley (1960), Rogers (1960), Evans and Karnovsky (1961), and Spector and Willoughby (1963). Reviews of phagocytosis have been published by Hirsch (1965) and Aarum (1967).

The mechanism of intracellular degradation of phagocytized microorganisms has been described in general terms for the vertebrates (Figure 2). Lysosomes—granules in the cytoplasm of phagocytes—contain antibacterial substances and hydrolytic enzymes (Cohn, Hirsch, and Wiener, 1963). The lysosome membrane

fuses with the vacuolar membrane within the phagocyte, releasing antimicrobial components into the vacuole (Robineaux and Frederic, 1955; Hirsch, 1965; Aarum, 1967). Evidence for comparable intracellular events in invertebrates is sparse, but Janoff and Hawrylko (1964) reported lysosomal enzymes in clams and starfish, and Eble (1966) found hydrolytic enzymes in oyster phagocytes.

Invading microorganisms are subjected to antimocrobial factors both inside and outside the phagocytes. Substances of presumed cellular origin, such as lysozyme, occur in the phagocytes and the plasma. A great array of such antimicrobial factors was identified in vertebrates (Skarnes and Watson, 1957; Elberg, 1960; Hirsch and Cohn, 1960; Landy, 1960; Coombs, Coombs, and Ingram, 1961; Mackaness, 1962; Miles, 1962), and some counterparts were recognized in invertebrates. McDade and Tripp (1967), for example, reported lysozymes in oyster hemolymph.

In the vertebrates, specific and nonspecific serum proteins increase the speed and effectiveness of phagocytosis—the opsonizing effect (Wright and Douglas, 1903; Suter, 1956; Rowley, 1960). Sensitization of bacteria with serum factors is not always a necessary prelude to phagocytosis, however, as was pointed out by Wood, Smith, and Watson (1946) and Wood (1953). In the absence of other host responses, early phagocyte activity may be important in preventing infection.

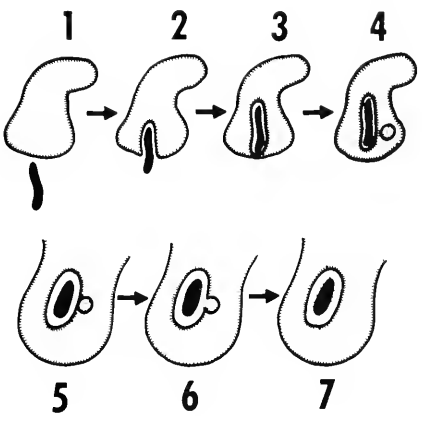

FIGURE 2.—Role of cell membrane and lysosome breakdown in phagocytosis. 1-4; phagocytosis; 5-7: lysosome activities. (Redrawn from Hirsch, 1965.)

Phagocytosis, then, constitutes the keystone to resistance. As Aarum (1967) mentioned, "... the organism's ability to oppose infection precisely follows the phagocytes' ability to function optimally. Resistance is lowered by a lowering of phagocytic activity." Phagocytosis can occur at the site of a lesion, in the filtering tissues and organs of the circulatory system, and (to a lesser extent) in the body fluid itself. Groups of fixed phagocytic cells are present in many crustaceans, most commonly in the sinuses and lacunae of the gills and at the bases of the legs.

Phagocytes agglutinate, aggregate, and cooperate in defense—forming nodules (the "nodules leucocytaires" of Cuénot, 1898), which are also known in annelids, mollusks, echinoderms, and other invertebrates. In a number of animals the nodules are brown, due to presence of large numbers of brown granules in the phagocytes, which may be excretion products or decomposition products. In *Gammarus*, the phagocytes composing the nodules secrete a clear yellowish chitinoid substance around the parasites. This secretion gradually becomes dark brown. The nodules appear as conspicuous black spots in infected individuals (Pixell-Goodrich, 1928) and are found frequently in gills and appendages. It should be clearly understood, however, that there are few detailed modern studies of phagocytosis in crustaceans or other invertebrates. Data from in vitro studies are particularly scarce, so there should be no implication that the kinetics, energetics, or other aspects of phagocytosis are fully understood.

Hemocytes of crustaceans and other invertebrates also act in other ways to protect the individual from overwhelming microbial invasion. Hemocytosis and hemocytic infiltration have been described in a number of invertebrate groups. Manifestations in invertebrates and vertebrates involve proliferation of hemocytes, changes in permeability of blood vessels, leakage

of blood fluids into tissues, adherence of hemocytes to blood vessel walls, and migration of hemocytes into tissues around areas of injury or parasitic invasion.

The involvement of hemocytes in coagulation or clot formation is a complex one, inasmuch as either cellular or extracellular clots may be formed. Intravascular cellular clots adhere to walls of blood vessels and spaces, producing stasis, and once they are formed, persist for some time. Extracellular clots, resulting from release of constituents of hemocytes, can inhibit microbial motion and thus render microorganisms more vulnerable to phagocytosis. Since Fredricq (1879) first pointed out that in Crustacea coagulation of hemolymph involves cell agglutination as well as plasma coagulation, others have demonstrated similar characteristics in a number of invertebrate groups. The release of a component from hemocytes and the role of this component in initiating coagulation of plasma were reported by a number of authors beginning with Halliburton (1885). Löwit (1889) observed the rapid disruption of hemocytes and the rapid clotting characteristic of most Crustacea and concluded that a causal relation existed. Hardy (1892), Tait and Gunn (1918), Tyler and Scheer (1945), and George and Nichols (1948) all provided data which supported the conclusion that a component from certain hemocytes acts with fibrinogen of plasma to form fibrin clots.

Bang (1967c, 1968) demonstrated that in the hermit crab, *Eupagurus longicarpus*, clots formed in at least two stages following injury—first a clumping and stickiness of hemocytes without change in shape or loss of granulation, then retraction of the clot and the development of a network of fibrous cell projections containing microtubules.

The abundant and elaborate literature on hemolymph coagulation in Crustacea was admirably summarized and evaluated by Florkin (1960). As he pointed out, coagulation has been considered by some authors to occur in two distinct phases—cellular coagulation and then plasma gelation—while other workers view coagulation as a continuous process in which plasma gelation begins around hemocytes. It was Florkin's conclusion that plasmatic coagulation was a one-step process in which fibrinogen of the plasma was acted upon by a coagulin released by the hemocytes. It seems equally possible, however, that more than one type of coagulable protein exists and that the categories of clots may be complex rather than simple. Florkin also reviewed the role of cellular clots in wound repair, emphasizing the importance of secretion of a chitin film over the wound area by underlying coagulated phagocytes.

Encapsulation is also a common form of cellular internal protection in invertebrates. Invading organisms, often relatively large, are surrounded by phagocytes and fibrocytes. The onset of encapsulation may be rapid, and the cellular aggregates may be resolved only very slowly.

In summary, the hemocytes function in a number of ways beyond phagocytosis, although the latter must be considered the dominant cellular defense mechanism:

1. Hemocytes are important in cellular infiltration of injured or diseased tissue.
2. They are important in clotting—either as participants in cellular clots, or by release of secretions or injury products which combine with plasma components to form extracellular clots.
3. The hemocytes are of primary importance to encapsulation.

A great variety of crustacean hemocytes have been described during the past several decades. Animals studied included crayfishes (George and Nichols, 1948; Toney, 1958; Wood and Visentin, 1967), blue crabs (George and Nichols, 1948; Toney, 1958), lobsters (Toney, 1958; Hearing and Vernick, 1967), and brine shrimp (Lochhead and Lochhead, 1941). Except for size differences, the principal distinction seemed to be presence or absence of granules in the cytoplasm. The hyaline hemocytes are usually smaller than the granular, and some of the hyaline cells probably develop into the granular types, since the intergrades have been noted (Cuénot, 1895). As was aptly pointed out by Rabin (personal communication), "The developmental relationships of one form of hemocyte to another which have been made amount to little more than edu-

cated guesses, since they have been based largely on static images which may not even represent the true cell pictures as they occur *in vivo*." Both types of cells (hyaline and granular) probably have physiological subtypes, as suggested by the work of Fisher-Piette (1931) in which explants of lobster hemopoetic tissues resulted in multiplication of two types of hyaline cells—adhesive ameboid and non-adhesive non-ameboid. Inclusions of granular cells, in addition to their defensive function mentioned earlier, may also provide nutrient material—as suggested by release of this material into the hemolymph during ovarian development (Lochhead and Lochhead, 1941). Hyaline hemocytes may also be transformed into connective tissue or endothelial cells of blood vessels (Danini, 1925, 1927; Debaisieux, 1952a, 1952b; Demal, 1953). Hemocyte physiology and biochemistry are areas where additional studies are needed, but the extreme fragility of certain cells once they are removed from the normal animal has undoubtedly been a major deterrent.

Phagocytic activity has been ascribed in varying degrees to most recognized categories of hemocytes (Haeckel, 1862; Hardy, 1892; Cuénot, 1895; Bruntz, 1905, 1907; Kollman, 1908; Tait and Gunn, 1918; George and Nichols, 1948; Toney, 1958; Rabin, 1970b).

In recapitulation, the phagocytes of vertebrate and invertebrate animals have been investigated widely since the late 19th century, and the blood cells of Crustacea have received at least proportionate study. Cellular defenses of Crustacea and other invertebrates are varied but center on the phagocyte and its activities. Important also are the humoral defenses, which will be considered in the following sections. Before proceeding to considerations of other than cellular defenses, however, it seems relevant to include an often overlooked perimeter of defense suggested by Miles (1962). Early suppression of microbial numbers may be due to microbicidal activity of the tissue cells themselves, either innate or induced, or to soluble antimicrobial substances in the intercellular fluid of the integument. As Miles pointed out, such pre-inflammatory cellular defenses in no way diminish the importance of phagocytes and humoral factors, but only provide an added perimeter of defense. Antimicrobial capacities of tissues as a whole, and of nonphagocytic cells in particular, may be a mainstay of nonspecific resistance—in both primary invasion and the determination of subsequent courses of infection. Tissue defenses of this nature in the invertebrates may be of great significance.

HUMORAL DEFENSE SYSTEMS

Early studies of humoral factors in Crustacea produced significant, but at times ambiguous, results. Noguchi (1903) found that sera of lobsters and horseshoe crabs possessed natural agglutinins against various vertebrate erythrocytes. After repeated injections, he was able to demonstrate an induced hemolysin in the horseshoe crab but not in the lobster. Fredericq (1910), using a variety of antigens, was unable to demonstrate precipitins in a number of decapods (*Homarus vulgaris*, *Palinurus vulgaris*, *Carcinus maenas*, *Portunus puber*, *Cancer pagurus*, and *Astacus fluviatilis*).

The early literature on humoral mechanisms of internal defense in invertebrates, and especially the crustaceans, was clearly dominated by Cantacuzène and his students. Cantacuzène, in a period covering almost 3 decades beginning in 1912, examined the broad picture of humoral defences of a large number of marine invertebrates. His work with Crustacea will be summarized in the next few pages as background for a consideration of subsequent studies.

Cantacuzène (1912a) reported (in a paper consisting essentially of a series of statements but little supporting data) the presence of natural agglutinins, lysins, and precipitins in serum of the hermit crab, *Eupagurus prideauxii*. Hemolysins for sheep and rabbit erythrocytes occurred in crab serum to a maximum titer of 250 and were destroyed by heat at 55° C. Agglutinins for rabbit erythrocytes persisted in dilutions beyond those at which hemolysis disappeared. Agglutinins for bacteria (*Escherichia coli* and *Vibrio cholerae*) were also present, as were weak precipitins against horse and rabbit sera. Cantacuzène also stated that comparable agglutinins, lysins, and precipitins were not present in *Pagu-*

rus striatus, which is closely related to *Eupagurus prideaux*ii.

Cantacuzène (1923b), summarizing a decade of study of humoral defenses in invertebrates, found that the serum of the spider crab, *Maia squinado*, possessed natural agglutinins for mammalian red blood cells, with great individual variation from crab to crab. He observed, rather significantly, that agglutinins weakened or disappeared completely in crabs held in captivity for long periods. He noted, on injection of erythrocytes, that agglutinins disappeared completely during the first days after inoculation and did not return to their original titer for several weeks after the last dose. Cantacuzène also reported that rare individuals of *Maia*—invariably moulting females—possessed a lysin for mammalian red blood cells. *Maia* serum also possessed a strong lytic factor, but no agglutinins, against cholera vibrios.

Cantacuzène inoculated *Maia* with coelomic fluid of *Sipunculus nudus* (4 to 5 injections at intervals of 3 to 5 days) and found that the crabs produced first agglutinins and then lysins against the various injected sipunculid coelomic cells, including ova. The nature, intensity, and duration of response varied greatly among individuals. The lytic ability seemed more pronounced in females than in males, and more so in females approaching sexual maturity. Hemolysins against mammalian erythrocytes were also produced.

When he compared the lytic ability of *Maia* serum after injection with sipunculid fluid and mammalian erythrocytes, Cantacuzène found that response was more rapid and stronger with the former, and he concluded—on the basis of these and other studies—that mammalian red cells were only mediocre antigens for marine invertebrates. He attributed this weak antigenicity to coating of the injected mammalian erythrocytes in *Maia* and other invertebrates with a serum factor that interfered with subsequent reactions. Cantacuzène aptly referred to this as "mummification" of the red cells by invertebrate body fluids.

Concerning acquired immunity to bacterial infections in Crustacea, Cantacuzène (1923b) found that the crab *Maia squinado*, when inoculated with killed *Vibrio cholerae* or small doses of live vibrio, was able—12 days after the fifth injection—to survive the challenge with 20 times the dose of vibrio which had proved fatal to uninoculated control crabs.

Cantacuzène also reported on studies of the responses of *Maia squinado* to injections of gram-positive bacteria isolated from the crab's digestive tract. Inoculation was followed by reduction in numbers of amebocytes and, within 24 hr, by disappearance of most of the bacteria from the hemolymph. The bacteria were immobilized in the various phagocytic tissues, particularly in the branchial lacunae—at first they adhered to the cell surfaces, then amassed into small granules, and were finally engulfed by fixed and mobile phagocytes. The process of digestion of bacteria was slow, and still incomplete after 7 weeks. Clotting ability of the hemolymph decreased immediately after inoculation but returned to normal in 8 days. No agglutinins for the bacteria could be demonstrated in vitro, but the natural agglutinins for mammalian red blood cells (discussed earlier) disappeared. In vitro studies disclosed that the hemagglutinin coated the bacteria but did not cause their agglutination. The adherence in vivo of the bacteria to fixed phagocytic cells undoubtedly was enhanced by the sensitization. Some evidence for this was gained by adding macerated hypodermal or pericardial cells to a mixture of bacteria and crab serum in vitro. The cell fragments acted as centers for bacterial agglutination and immobilization, but the agglutinating ability was not conferred on the serum by the addition of cell fragments.

A different sequence of events was described for those crabs (*Maia*) in which the experimental infections progressed to death. Early immobilization of bacteria in lacunar cells was followed, in 8 to 15 days, by the appearance of encapsulated forms, invulnerable to destruction by phagocytes. The encapsulated bacteria multiplied, phagocyte numbers were reduced, and the clotting ability of the hemolymph diminished. By 10 to 20 days after inoculation, the hemolymph became incoagulable, the natural agglutinin for red cells disappeared, the connective tissue became gelatinous, and the animal died.

Cantacuzène (1923b) also examined the internal defenses of the hermit crab, *Eupagurus prideauxii*, which he had reported earlier to possess strong hemolysins and strong antibacterial agglutinins. Injected gram-negative bacilli were entrapped and immobilized on the cell surfaces of the branchial lacunae, and then phagocytized, as was the case with *Maia* described previously.

Cantacuzène (1923b) also reported that the sera of crabs, *Carcinus maenas*, infected by the rhizocephalan *Sacculina*, contained a factor absent from normal crabs. Using a standard complement fixation test, with extract of the rhizocephalan as antigen and with crab serum, Cantacuzène (1925b) was able to demonstrate an antibody-like response in parasitized crabs. Sheep cells were lysed in tubes with normal crab serum but not in those containing parasitized crab serum. Using a fine suspension of *Sacculina*, Cantacuzène found precipitating and agglutinating activity in the serum of parasitized crabs. The activity was not consistent, however, in that some sacculinized crabs lacked it. In a concurrent study, Lévy (1923) found that macerated sacculinids were toxic when injected into crabs, but that no antitoxic activity could be demonstrated in parasitized crabs. Both groups, normal and parasitized, died at about the same rate.

In an earlier study Cantacuzène (1913) reported that inoculation of the sacculinid parasite with gram-negative bacteria resulted in septicemia and death of the parasite within 1 week, and infection of the crab host by 10 days after inoculation. At about 5 days after inoculation, the crab hemolymph became incoagulable, and agglutinins appeared against the bacteria inoculated into the parasite. The antibacterial agglutinins were not present in sacculinized uninoculated control crabs.

Appreciable evidence for some degree of specificity of the natural agglutinins of Crustacea was accumulated by Cantacuzène. An agglutinin in *Maia* against mammalian red cells could be absorbed from crab serum by certain gram-positive bacteria but did not agglutinate them, nor did it agglutinate cholera vibrios. It did, however, strongly agglutinate typhoid bacilli. Agglutinins against vertebrate erythrocytes and certain bacteria were found in sera of *Homarus vulgaris*, *Eupagurus prideauxii*, and *E. bernhardus*, but the same antigens were not agglutinated by sera of *Cancer pagurus*, *Carcinus maenas*, *Portunus puber*, or *Galathea punctata*. The serum of *Eupagurus prideauxii* agglutinated mammalian red blood cells and amebocytes of *Maia* and *Buccinum*, but did not agglutinate the coelomic cells of sipunculids or ascidians.

And so Cantacuzène set the scene, by the early 1930's, for the continuation of broad and elaborate studies of humoral internal defenses of marine invertebrates—particularly the Crustacea—but the stage, with only a few notable exceptions, remained curiously empty and dark until the mid-1950's. Although research during the last decade emphasized species other than those studied by Cantacuzène (except for the work of F. Bang), it reinforced many of Cantacuzène's findings: that natural agglutinins and lysins, with some specificity, occur in Crustacea and other invertebrates, and that responses to foreign antigens can be induced in selected invertebrates—responses which are only partially specific. The increased precision and quantitation of tests, and the careful attention to controls, have improved the quality of the newer data, but have neither provided new concepts nor modified the general conclusions of Cantacuzène. The more recent literature on humoral defenses of Crustacea will be summarized by general categories in the following sections.

Bactericidal Systems

Natural bactericidins have been reported from a number of marine invertebrates (Bang, 1967b). Recently, increase in titers of bactericidal activity after inoculation with Formalin-killed bacteria was noted in West Indian spiny lobsters, *Panulirus argus*, and American lobsters, *Homarus americanus* (Evans et al., 1968; Acton, Weinheimer, and Evans, 1969). A bactericidal assay system described by Schwab and Reeves (1966) was used to quantitate the degree of response. In experiments with American lobsters held in seawater at 5° C, the peak of

bactericidal response against gram-negative bacilli was reached within 48 hr after inoculation, and high titers persisted throughout the observation period (11 days). In spiny lobsters, presumably maintained at significantly higher environmental temperatures at Bimini, the Bahamas (reported as 26°-28° C in a later paper), the primary bactericidal response to intracardial injection of living or killed suspensions of the same gram-negative bacilli (originally isolated from the digestive tract of spiny lobsters) reached a peak at about 36 to 48 hr after injection, then declined slowly for the following 2 weeks. Partial lack of specificity of the bactericidin was indicated by its appearance following injection of gram-positive bacilli and by its activity against *Salmonella typhosa* and *Escherichia coli*, as well as against the unidentified gram-negative bacillus used as the homologous test organism. The bactericidin was not active, however, against *Pseudomonas aeruginosa* or against three species of gram-positive bacteria.

A subsequent study (Weinheimer, Acton, Sawyer, and Evans, 1969) of the specificity of the spiny lobster bactericidin further indicated that the response was partially nonspecific. Low titers of the bactericidin against gram-negative bacilli could be demonstrated after injections of Formalin-killed type 2 pneumococci and bovine serum albumin. Formalin produced a pronounced adjuvant effect.

Secondary responses—those following reinjection of the same antigen after a lapse of time—of spiny lobsters to killed suspensions of gram-negative bacilli were also examined by Evans, Cushing, Sawyer, Weinheimer, Acton, and McNeely (1969). Titers of bactericidin were slightly but significantly higher after reinoculation than after primary inoculation, and the rate of secondary response (the number of hours to reach peak titer) seemed somewhat accelerated when compared with the primary response. Unfortunately, the number of animals tested was small. It seems important that similar observations be greatly extended—since, as the authors pointed out, the results were reminiscent of the specific anamnesis or immunological memory demonstrable in the immunoglobulin responses of vertebrates.

The spiny lobster bactericidin was apparently a large molecule, as suggested by resistance to dialysis and by Sephadex separations. Inactivation occurred at 65° C and activity was not restored by addition of unheated normal hemolymph. Activity was not reduced by treatment with EDTA or carageenin. These results indicate dissimilarity with vertebrate complement-based bactericidal systems, but the authors suggested that the bactericidin may represent a primordial immunoglobulin.

Noteworthy is that all the American lobsters and a number of the West Indian spiny lobsters used in the studies by Evans, Weinheimer, Painter, Acton, and Evans (1969) had demonstrable pre-existing titers of bactericidins against the gram-negative bacillus used in the experiments. Possibly the remainder of the spiny lobsters used in the studies could have had titers of bactericidins lower than were demonstrable by the methods used. Thus inoculation may not have "induced" the bactericidin but instead may have merely enhanced or increased the titers. A number of explanations were offered for the pre-existing titers of bactericidal activity, including trauma because of handling, and response to previous bacterial infection. Two relevant observations are that in many American lobsters, a rapid increase in bactericidal titer preceded death, and in studies of spiny lobsters, the bactericidal activity was found to be partially nonspecific, in that activity against other gram-negative bacteria was enhanced.

The conclusions reached by the research group that examined the spiny lobster bactericidin (Weinheimer, Acton, Sawyer, and Evans, 1969) are in accord with findings for other phyla: "These data suggest that, although invertebrates appear capable of antigenic recognition, the molecules synthesized may have broad specificity covering a wide range of antigenic determinants. Further studies will be necessary to ascertain whether these inducible substances represent primitive immunoglobulins. In any event, it would be surprising if they did not have a major role in defense of the animal against pathogenic microbes."

An important qualification was pointed out by Aarum (1967) regarding results obtained by ex-

perimental inoculation of large numbers of bacteria. Infectious agents are found in large numbers in the circulatory system only in septicemia, which occurs only after a growth phase of a virulent pathogen within the host, often within fixed or immobilized phagocytic cells. Rapid increase in pathogen numbers in the circulating body fluid is usually a precursor to death and indicates a failure of host defenses. Thus the artificial introduction of large numbers of microorganisms should not be expected to elicit normal responses in an animal. It should also be noted that insusceptibility barriers to infection, operative in the normal animal, may be bypassed by experimental inoculation, resulting in massive infection and death from microorganisms not otherwise known as pathogens. A further qualification mentioned by Aarum is that even the simplest experimental manipulation may change phagocytic abilities, so that the normal phagocytic response of an animal to pathogens may be far different from responses elicited experimentally.

Lytic Systems

A natural hemolysin for sheep erythrocytes in the hemolymph of West Indian spiny lobsters was reported by Weinheimer, Evans, Stroud, Acton, and Painter (1969). Low temperatures (0° and 4° C) inhibited lysis, which was best demonstrated at 25° and 37° C. Heating the lobster hemolymph to 52° C destroyed lytic activity. The hemolysin could be adsorbed on red cells or cell stroma at low temperatures. Red cells with the adsorbed lysin were lysed when the temperature was raised to 37° C, which indicated a possible enzymatic type of activity of this hemolytic system. The authors suggested a multiple step system, analogous to mammalian hemolytic systems, consisting of a single protein species which is first adsorbed on the surface of the sheep erythrocyte, and then—in one or more steps—lyses the cell.

A detailed study of the presence or absence of natural immunity to invasion of the hemolymph of crabs by a parasitic ciliate, *Anophrys sarcophaga*, was reported by Poisson (1930). The ciliate, which in shore crabs, *Carcinus maenas*, multiplies and causes death, was immobilized, agglutinated, and lysed in vitro by serum of several other crabs—particularly *Maia squinado*, *Eupagurus prideauxii*, and *Portunus puber*. Experimental inoculations of ciliates were cleared usually within several hours. It is interesting that the parasite, when experimentally introduced in another member of the Portunidae, *Portunus depurator*, multiplied and killed the experimental hosts just as it did in *Carcinus maenas*. Poisson attributed the reactions of crabs to the ciliate to expressions of a natural immunity, effected by agglutinating and lytic activity of the hemolymph.

Other lytic systems of Crustacea have received passing attention. Cantacuzène (1913) found that hemolymph of the hermit crab, *Eupagurus prideauxii*, possessed a heat-labile hemolysin, as well as precipitating and agglutinating activities. Cantacuzène (1921, 1923b) stated that injection of the spider crab, *Maia squinado*, with sheep erythrocytes produced hemolysins as well as agglutinins.

Agglutinating Systems

Natural hemagglutinins of the Australian freshwater crayfish, *Parachaeraps bicarinatus*, were examined by McKay, Jenkins, and Rowley (1969). Absorptions of hemolymph by erythrocytes of four mammalian and one avian species disclosed specificity, in that absorption by cells of one species still left agglutinins for cells of other species. The crayfish hemagglutinins were nondialysable and were inactivated at 57° C. In vitro studies with crayfish phagocytes and mouse erythrocytes disclosed that the crayfish hemagglutinins greatly enhanced the adhesion of the erythrocytes to the phagocytes—a specific opsonic effect. A similar effect was observed in vivo, apparently as a prelude to phagocytosis.

The recent studies support earlier reports of specific hemagglutinins in a number of invertebrate phyla (Tyler and Metz, 1945; Tyler, 1946; Sindermann and Mairs, 1959; Cushing, Calaprice, and Trump, 1963; Tripp, 1966) with specificities somewhat comparable to those of vertebrate natural isohemagglutinins and with some biological properties (such as enhancement of

adhesion and phagocytosis of erythrocytes by phagocytes) similar to those of vertebrate antibodies.

A study of natural agglutinins in the serum of California spiny lobsters, *Panulirus interruptus*, for blood and sperm cells of 54 species (representing 7 phyla) was published in 1945 by Tyler and Metz, and is still cited as one of the most comprehensive examinations of its kind for a marine animal. Agglutination was not found in 37 other species tested. Titers for positive reactions ranged from 8 to 256. The most interesting phase of the study was an extensive series of absorptions of spiny lobster serum by cells of many of the species tested. In each case, absorption of serum with cells of any single species removed agglutinins for all the species tested that belonged to the same group (Class) but left agglutinins for the cells of all other groups tested. Tyler and Metz concluded, on the basis of absorptions, that at least 10 class-specific agglutinins were present in the serum of the spiny lobster. A few cross-reactions occurred, and some reduction in titers resulted from absorptions with cells of other species, which suggested the presence of a number of reacting sites on the cells.

Bang (1967b) examined the responses of the spider crab, *Maia squinado*, to injections of *Anophrys*, a large ciliate pathogenic for shore crabs, *Carcinus maenas*, as part of a laudable attempt to repeat with modern methods some of the early French studies of invertebrate defenses. Sera of some spider crabs strongly agglutinated the ciliates, but that of others did not. Agglutination resulted from formation of a mucoid substance around the tail cilia. Crabs that lacked the agglutinin died from overwhelming infections of the hemolymph, whereas those with the agglutinin survived (Bang, 1962). When present, the agglutinin was apparently fairly constant, except that in some crabs it was lost spontaneously but temporarily at time of molting. Poisson (1930) had noted earlier that the hemolymph of *Maia* lysed the ciliates and that the hemolymph of the hermit crab, *Eupagurus prideauxii*, agglutinated them.

An agglutinin in the hemolymph of the hermit crab, *Paguristes ulreyi*, for human type O cells, and to a lesser extent for types A and B, was reported by Cushing (1967). A number of individuals lacker the hemagglutinin, and some of these possessed a serum factor which inhibited the action in vitro of the positive sera. The analogy to specific soluble substances found in sera of certain vertebrates was pointed out by Cushing, with the suggestion that further studies of this kind with other invertebrates might prove instructive. Cohen (1968) found agglutinins for human and other vertebrate erythrocytes in sera from coconut crabs, *Birgus latro*. Young crabs lacked the agglutinins.

The serum of the American lobster contains strong and specific natural agglutinins for an antigen present on the red blood cells of sea herring, *Clupea harengus* (Sindermann and Mairs, 1959). Detection of blood groups in this fish was aided by the use of the hemagglutinin—individual herring either had the antigen or lacked it. Titers reached as high as 256, and reactions paralleled those obtained with absorbed rabbit antisera and plant lectins (Sindermann, 1963). Erythrocytes of other clupeoid fishes tested were also strongly agglutinated by lobster sera (Sindermann, 1962).

Precipitating Systems

Production of precipitins by invertebrates has been reported only rarely. Osawa and Yabuubhi (1963), in a very brief paper, found that the "Homard americain, *Cambarus clarkii*" [probably *Homarus americanus*] did not produce agglutinins or lysins when injected with red blood cells but did produce weak precipitins (detectible by immunoelectrophoresis) after injection with serum from rabbits and goats. No information was given about dosage, injection schedules, time for response, or titers.

Stewart and Foley (1969) suggested that a "precipitin-like principle already present in the hemolymph" of the American lobster might be important in removal of foreign protein. Fluorescein-labelled bovine serum albumin (BSA) and lobster serum proteins were injected into lobsters held at 5° C, and the fluorescence level checked periodically for 6 days. With lobster serum proteins, an initial decline in fluorescence

within 2 hr after injection was followed by a plateau that remained constant for the duration of the experiment; with labelled BSA, however, the rate of clearance was concentration dependent, but the foreign protein declined to very low levels by 3 days after injection. Fluorescent material was apparently excreted in proportion to its disappearance from lobster hemolymph. Pinocytosis by phagocytes was not demonstrated, but tiny granular fluorescent accretions were observed in the hemolymph of lobsters injected with labelled BSA, beginning about 8 hr after injection. In vitro studies, in which a standard ring test with labelled or unlabelled BSA and lobster serum was used, disclosed a clearly discernible ring after 16 hr at room temperature, and a precipitate at the bottom of the tube after 36 hr. Precipitin titers of individual lobster sera ranged from 2 to 16 and were independent of the total protein concentration of the lobster serum. Dialysis of lobster serum did not change the titers, but precipitin activity was destroyed by heating above 50° C.

A number of interesting implications in the study were pointed out by Stewart and Foley. The clearance mechanism seemed able to distinguish between foreign and native proteins, and the capacity for clearance seemed high, as indicated by accelerated clearance of larger doses of BSA. Attempts to increase the levels of precipitin by previous injection of lobsters with BSA did not succeed, and in fact resulted in decreased precipitin levels in some individuals. The authors suggested that the hemolymph factor responsible for clearance of foreign protein may be maintained normally at low levels and may be supplemented by further secretions when required. They also suggested that the precipitin principle in the hemolymph may be the first of several steps or possibly the primary removal factor and that digestion and excretion may take place elsewhere—probably in the hepatopancreas.

The results of the studies of Stewart and Foley are in agreement with those of Teague and Friou (1964), who observed that injected foreign protein was rapidly removed from the hemolymph of the crayfish *Cambarus virilis*. Previous injection of the protein did not increase the clearance rate. Teague and Friou did not observe precipitin activity against injected bovine and human serum albumins but concluded that clearance resulted from nonspecific degradation of the foreign protein.

Other evidence of clearance of injected proteins but failure to induce heightened responsiveness in Crustacea was reported by Campbell and Garvey (1961). They mentioned that "It is also of interest that we have made many attempts to induce antibody formation in invertebrates, e.g., lobsters. We have been unsuccessful so far, but in every instance the antigens remained undigested and unchanged in the circulation and tissues for many months." Although not mentioned specifically, the lobsters were probably California spiny lobsters and the test antigens probably included BSA, since this was the principal antigen used in other studies reported in the same paper.

Phage Clearance

Taylor, Taylor, and Collard (1964) and Nelstrup, Taylor, and Collard (1968) presented some evidence (from two crabs) of an increase in the rate of secondary clearance of injected T_1 bacteriophage in the shore crab, *Carcinus maenus*. Clearance was not complete until after 2 weeks at 16° to 18° C, and no neutralizing antibody to T_1 phage was detected in the hemolymph. Primary inoculation with T_6 phage did not increase clearance rates for T_1 secondary injections. The small number of animals used in these experiments makes the conclusions highly tentative. The authors suggested the existence of a "phylogenetically more primitive type of immune response than the production of humoral antibody," but did not state clearly what the response was—except possibly that it was "an apparently purely cellular secondary response."

Studies of phage clearance by Cushing and McNeely (reported in Cushing, 1967) led to negative conclusions. Phage T_4 persisted for up to 168 days in the California spiny lobster and disappeared at a steady rate, uninfluenced by the size of the original inoculum. Two species of crabs tested also failed to clear bacteriophage.

Other negative findings for increased rate of phage clearance following inoculation in crayfish were reported by Teague and Friou (1964).

Antitoxic Activity

Little definitive information is available about antitoxic activity in invertebrates. As Huff (1940) pointed out, "Experimental demonstration of antitoxic action in invertebrates has failed for the most part because of lack of susceptibility of invertebrate cells for known toxins." Probably the best example of antitoxic phenomena in Crustacea was described by Cantacuzène (1925a) and Cantacuzène and Damboviceanu (1934a, 1934b). The hermit crab, *Eupagurus prideauxii*, exhibited resistance to nematocyst toxin of *Adamsia palliata*, a commensal coelenterate commonly found on the shell of the crab. When injected, the toxin had no effect on *E. prideauxii*, but it was lethal to many other Crustacea and to a number of other invertebrates tested, including the closely related hermit crab, *E. bernhardus*. Cantacuzène also found that serum of *E. prideauxii* could neutralize the coelenterate toxin when the two —serum and toxin—were mixed and injected into crab species susceptible to the toxin. The development of this antitoxic principle can be seen as a logical and necessary concomitant of the very close relationship of crab and anemone, but the question of whether this is an example of innate or acquired resistance has not been resolved.

Another example of coelenterate toxin lethal to crabs was reported by Lane, Coursen, and Hines (1961). Biologically active peptides in *Physalia* nematocyst toxins were tested, using fiddler crabs, *Uca pugilator*, as assay animals.

Except for the work with coelenterate toxins, evidence of antitoxins in invertebrates is weak. Stauber (1961) reported almost immediate removal of diphtheria toxoid from oyster blood, but Metchnikoff (1905) and Bengston (1924) found that tetanus and botulinus toxins remained in insect body fluids for several weeks without loss of toxicity. These studies must, of course, be viewed as most indecisive, since substances toxic to humans are not necessarily so to invertebrates. Reaction on the part of invertebrates could be identical to reaction against any other introduced foreign material.

Invertebrate responses to gram-negative bacterial endotoxins were the subject of a review by Levin (1967). The most striking activity of such endotoxins is the production, after experimental inoculation, of intravascular clots and the ensuing death of various crustaceans and other invertebrates. Antitoxic immunity has not been demonstrated, but, as Levin stated: "Endotoxin appears capable of activating complementary defense mechanisms in invertebrates, including aggregation of amoebocytes, coagulation, bacterial immobilization, and phagocytosis. All these may be operative through one type of cell—the amoebocyte."

Other Protective Systems

McKay and Jenkin (1969) examined resistance of the Australian freshwater crayfish, *Parachaeraps bicarinatus*, to a pathogenic *Pseudomonas* sp. and concluded that the animal was capable of an adaptive immune response. Their findings indicated lower mortality rates (after bacterial challenge) in animals inoculated with heat- and alcohol-killed vaccines as well as with endotoxin. Inoculation with vaccines prepared from other gram-negative bacteria also increased the level of resistance to the *Pseudomonas* infection, but vaccines from gram-positive bacteria did not—indicating some degree of specificity. A positive correlation was found between survival of challenged animals and the number of exposures to bacterial antigen; after four inoculations, the LD_{50} of immunized animals was nearly 100 times that of controls. Temperature also played a significant role in the onset, degree, and duration of protection induced by inoculation of animals with killed bacteria. At 26° C, onset of protection was rapid (1 day), reached a peak at 3 days, and almost disappeared by 12 days; at 19° C, onset was slower (2 days), reached a maximum at 4 days, and persisted for 12 days (the duration of the experiment); at 11° C, no protection was afforded. Inoculation of gram-negative endotoxin resulted in protection similar in appearance and duration to that

produced by killed vaccines. Although the terms "immunity" and "resistance" were used, the precise nature of the protection afforded by inoculation of vaccines and endotoxin was not described by the authors. In vitro experiments with hemolymphs of control and resistant crayfish disclosed no bactericidal or bacteriostatic effects, and McKay and Jenkin suggested that the most important effect of immunization may have been to increase the metabolic rate of the phagocytes [thereby stimulating phagocytosis].

Barker and Bang (1966), extending the earlier studies of Cantacuzène (1925b) with the shore crab, *Carcinus maenas*, and its rhizocephalan parasite *Sacculina carcini*, reported that inoculations of *Vibrio* sp. caused the hemolymph of the parasite to become incoagulable within 24 hr. Masses of gelled material containing bacteria were seen within body spaces. Septicemia and death, first of the parasite, and then often of the crab host, followed soon after.

Insusceptibility factors seem operative when certain parasites of invertebrates fail to develop. Michajłow (1938) and Baer (1944), for instance, found that larval cestodes, *Triaenophorus* and *Ligula*, penetrated the intestinal wall of a number of copepods, but developed only in certain species. In others, the larvae died and were phagocytized. Hedrick (1935) observed similar differences in survival of larval nematodes. Léger and Duboscq (1908) reported earlier that sporogony of the sporozoan *Agregata eberthi* (which occurs in the intestinal wall of crabs of the genus *Portunus*) took place readily in all species except *P. puber*, in which the parasite was quickly phagocytized after invading the intestinal wall.

INTERNAL DEFENSE MECHANISMS INVOLVED IN GAFFKAEMIA OF LOBSTERS

The American lobster, *Homarus americanus*, has an effective internal defense system, consisting of active phagocytosis as well as agglutinating and bactericidal (or bacteriostatic) activity, against a number of injected bacteria. The protective system seems to fail completely only when challenged by *Gaffkya homari*—which is thus far the only bacterial pathogen known to develop systemic infections in lobsters and to kill them. Probably the most extensive series of reports concerned with responses of invertebrates to a particular pathogen is that dealing with the lobster (and other decapods) and the highly pathogenic gram-positive micrococcus *G. homari*. "Gaffkaemia"—the disease caused by *G. homari*—is enzootic in both the American lobster, *Homarus americanus*, and the European lobster, *H. vulgaris*, and has been reported to cause epizootics in captive populations of both species (Roskam, 1957; Goggins and Hurst, 1960; Gibson, 1961; Stewart and Rabin, 1970). Microorganisms with characteristics of *G. homari* have been isolated from shrimp (*Penaeus aztecus* from the Gulf of Mexico) and from crabs (*Carcinus maenas* and *Libinia emarginata* from New England and *Cancer irroratus* from eastern Canada), but the disease "gaffkaemia" is known only in lobsters. Early descriptions of the disease and its etiological agent (Hitchner and Snieszko, 1947; Snieszko and Taylor, 1947) have been followed during the past decade by studies in several laboratories, which used the lobster and the pathogen as a test system to elucidate responses to infection and other aspects of the host-parasite relationship. The possible course of infection in lobsters is summarized in Table 1. Snieszko and Taylor (1947) first satisfied Koch's postulates for the pathogen and demonstrated high mortality following inoculation of cultured *G. homari*. Stewart and MacDonald (1962) and Stewart et al. (1966) found that 40 to 60% of lobsters they examined from certain locations on the Canadian east coast were infected.

Studies by Harvey Rabin at Woods Hole and The Johns Hopkins University (Rabin, 1965; Rabin and Hughes, 1968) confirmed that lobsters inoculated with *Gaffkya* became septicemic within 2 days and died a few days later. Inoculation of gram-negative endotoxin 10 hr before exposure to the pathogen did not alter the course of infection. Prior inoculation of heat-killed *Gaffkya* cultures (24 hr before challenge) produced no protection.

In vitro studies with lobster serum as a culture medium disclosed that *Gaffkya* growth was

TABLE 1.—A proposed hypothesis to explain the course of *Gaffkya homari* infections in lobsters at 15° C.

Day	Development of gaffkaemia in lobsters
0	Bacteria gain access to tissues of lobsters as a result of injury which destroys the integrity of exoskeleton (or possibly the gut epithelium). Lobster hemocytes phagocytize *G. homari*
2	Phagocytized bacteria may multiply within hemocytes. Hemocytes containing engulfed bacteria lodge in capillary and locunar areas (heart, hepatopancreas, gills) of the lobster.
4	Hemocytes may be disrupted, releasing *G. homari* in hemolymph. This may result in rapid decrease in hemocyte numbers and logarithmic increase in bacterial numbers.
6	Hemolymph stimulates multiplication of released bacteria. Hemocyte numbers seem to be gradually reduced by continued phagocytosis and disruption of phagocytes.
8-10	Clotting mechanism (release of coagulin from hemocytes) is affected—possibly by reduction of hemocyte numbers—and clotting time is greatly prolonged.
12-14	Lobsters die from depletion of nutrient stores and utilization of this material by *Gaffkya*. Injured gaffkaemic lobsters may bleed to death. (The possibility of exotoxin has not been entirely eliminated, but there is no present evidence to suggest its existence.)

stimulated, while growth of a *Vibrio* (nonpathogenic to lobsters) was usually inhibited. Serum from lobsters which had been inoculated 24 hr earlier with killed *Gaffkya* still stimulated growth of the pathogen in vitro.

Rabin and Hughes (1968) tested resistance to *Gaffkya* in a variety of studies with lobsters and other marine arthropods. Findings with spider crabs (*Libinia emarginata*), rock crabs (*Cancer borealis*), and horseshoe crabs (*Limulus polyphemus*) were that most of the test animals cleared inoculated *G. homari*. In vitro studies with hemolymph disclosed either no apparent effect or only slight inhibition of growth of the pathogen by sera of spider and horseshoe crabs, and a slight stimulation of growth by sera of rock crabs.

The possible role of exotoxin was tested in lobsters by Rabin and Hughes with inoculation of filtrates of *G. homari* cultures. The filtrate had no effect when it was injected into the abdomen, but injection into the major joint of the chela induced autotomy or abnormal movements in over 50% of the lobsters treated.

Evidence of resistance to gaffkaemia was noted by Rabin and Hughes in a single lobster, which had been infected naturally before it was brought to the laboratory. Twelve days after capture the lobster was free of the pathogen. The animal was inoculated twice with increasingly larger dosages of *G. homari* and cleared the bacteria within 6 days—but died on the 11th day following the second challenge. The reactions indicated a partial resistance and an ability in some individuals to recover from gaffkaemia. It is interesting that serum from this presumably resistant lobster was similar to that of other lobsters tested in that it did not inhibit growth of *G. homari* in vitro.

Rabin and Hughes stated that the presence of *Gaffkya* infections did not damage the clotting mechanism—an observation quite different from that of Goggins and Hurst (1960), who found that reduction in amebocytes and a much prolonged clotting time were distinctive features of the disease. Stewart et al (1969) and Stewart and Rabin (1970) clarified these seemingly disparate observations by reporting that "coagulin" is released to initiate clotting by rupture of hemocytes and that the "concentration of plasma proteins, including fibrinogen, does not appear to decline significantly in gaffkaemic lobsters." An earlier report by Rabin and Hughes (1968) stated that when extract of lobster muscle was used as a coagulin source, recalcified clotting times were the same in diseased and normal animals. When these facts are combined, it can be concluded that the abnormally and persistently low hemocyte content of the hemolymph results in prolonged clotting time and does not indicate any deficiency in plasma constituents other than coagulin (Figure 3).

Studies carried on by James Stewart and his associates at the Halifax (Nova Scotia) Laboratory of the Fisheries Research Board of Canada have extended the work of Rabin and have provided the greatest number of contributions to the literature about the effects of *Gaffkya* disease on lobsters. In accord with earlier studies, infections usually were fatal, although rare individuals infected with *Gaffkya*-like organisms did survive (Stewart et al., 1966).

Cornick and Stewart (1968a) provided considerable relevant information about the host-parasite relationships of *Gaffkya* and lobsters. Experimental infections by inoculation, in which dosages as low as approximately 5 bacteria per

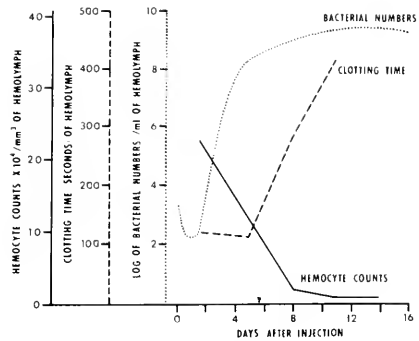

FIGURE 3.—Relations of hemocyte counts, clotting time, and bacterial numbers to time from experimental exposure of lobsters to *Gaffkya*. (Redrawn from figures in Stewart, Arie, Zwicker, and Dingle (1969) and Stewart and Rabin, 1970.)

lobster at 15° C were used, killed 90% of the test animals within 17 days. The absence of an effective host defense against *Gaffkya* was strongly indicated by the fact that the mean time to death was almost constant, regardless of dosage (Figure 4). Cornick and Stewart's studies disclosed additional facts that help to explain the pathogenicity of the bacterium to lobsters: *Gaffkya* resisted digestion in phagocytes and multiplied in the hemolymph; growth of *Gaffkya* was stimulated in vitro by serum of lobsters while growth of several other bacteria was inhibited; and *Gaffkya* was not agglutinated by lobster serum, though all other bacteria tested were agglutinated.

Presence in lobster hemolymph of effective defenses against bacteria other than *G. homari* was indicated by clearance within 30 days of inoculated suspensions of *Micrococcus conglomeratus*, *M. sedentarius*, *Achromobacter thalassius*, and *Gaffkya tetragena*. Since several of these bacteria are closely related to *G. homari* (which was not cleared), some specificity of the phagocytic or humoral protective mechanisms is strongly indicated.

Natural agglutinins in lobster serum were demonstrated against all bacteria tested (six genera), except for all strains of *G. homari*. Such agglutinins were of low titer, nondialysable, inactivated at 56° C, and seemed to be nonspecific (as suggested by the limited observation that a single absorption of serum by *Flavobacterium marinum* removed agglutinins for all other bacteria tested except *Brevibacterium* sp.).

Cornick and Stewart's observations on phagocytosis of *G. homari* are interesting and warrant further investigation. They found no phagocytized bacteria in hemolymph preparations 15 min after inoculation, but they did find fluorescent-dye-labelled bacteria in hemocytes in heart, liver, and gill tissues of experimental lobsters soon after inoculation. Circulating hemocyte numbers were reduced significantly within 15 min after bacterial inoculation but returned to normal levels after 5 hr. These data are in agreement with the statements of Maynard (1960), that phagocytes which have engulfed foreign material lodge in capillary and lacunar areas of the crustacean body, resulting in reduction in numbers of circulating hemocytes. Bang (1956) observed in tissues of *Limulus* injected with gram-negative bacteria a similar reduction in circulating hemocytes. In Cornick and Stewart's study long-term infections of lobsters were characterized by the presence of black nodules containing *G. homari* in tissue cells in the gills, swimmerettes, and ventral abdominal

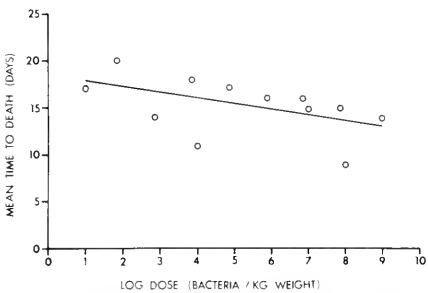

FIGURE 4.—Relation of dosage of *Gaffkya* to mean time to death (MTD) in lobsters (calculated line of best fit for mean time to death, using experimental groups of 10 lobsters each). (From Cornick and Stewart, 1968a.)

sinuses of the lobster. As Parry (1960) had pointed out earlier, this type of aggregation in gills is a common phenomenon in Crustacea. Poisson (1930) observed that, in the few crabs (*Carcinus macnas*) resistant to the parasitic ciliate *Anophrys sarcophaga*, masses of dead ciliates occurred in branchial lacunae, pericardial sinus, and hepatopancreatic sinuses. Degenerating ciliates eventually formed brownish cysts. Cantacuzène (1923b) observed a similar phenomenon in *Maia squinado* inoculated with bacteria. He pointed out that the lacunar tissue of the branchial lamellae of decapod crustaceans, with its many fixed phagocytes, acts as an extensive and effective bacterial filter.

Cornick and Stewart suggested that the development of polysaccharide capsules by *G. homari* in later stages of infection could be an important device that prevented destruction of the phagocytized bacteria and allowed multiplication of the pathogen. They pointed out, as evidence, that unencapsulated *G. homari* grown in culture were actively phagocytized. As Stewart and Rabin (1970) later reported, however, the unencapsulated cultured bacteria were also virulent. It may be that the capsule forms soon after the organisms are injected into the host. This observation of survival and growth of phogocytized encapsulated bacteria in lobsters is a direct counterpart of the inability of vertebrate phagocytes to destroy many encapsulated microorganisms, and parallels the earlier findings by Cantacuzène (1923b) of a fatal disease in crabs induced by encapsulated gram-positive bacteria.

Stewart, Dockrill, and Cornick (1969) examined certain insusceptibility factors affecting *Gaffkya* disease in lobsters. Destruction of the integrity of the integument seemed essential to transmission of the pathogen. Acidity of the gastric fluid was bactericidal and appeared to provide an effective barrier against oral infection. Previous attempts to infect lobsters by feeding infected material had been unsuccessful (Snieszko and Taylor, 1947; Wood, 1965a, 1965b; Rabin and Hughes, 1968).

Undoubtedly such insusceptibility factors are of definite importance to the epizootiology of gaffkaemia for several reasons: lobsters are cannibalistic; hemolymph of moribund gaffkaemic individuals contains about 10^9 organisms per ml (Stewart, Arie, Zwicker, and Dingle, 1969); and *Gaffkya* can be isolated consistently from lobster pounds, sea water, bottom mud, and slime of holding containers (Goggins and Hurst, 1960).

A thorough study of the effects of temperature on experimentally induced *Gaffkya* infections in American lobsters was reported by Stewart, Cornick, and Zwicker (1969). Mean time to death was inversely related to temperature (Figure 5). At 1° C no deaths attributable to experimental infections occurred; at 3° C mean time to death was 172 days; and at intermediate higher temperatures mean time to death decreased drastically to a minimum of 2 days at 20° C (which approaches the upper lethal tem-

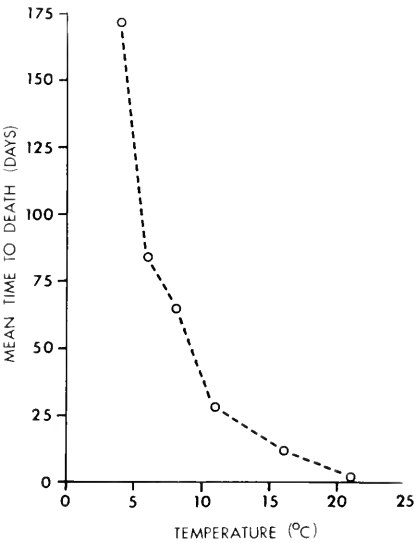

FIGURE 5.—Relation of temperature to mean time to death in lobsters experimentally exposed to *Gaffkya*. (From Stewart, Cornick, and Zwicker, 1969.)

perature for American lobsters). It is important to note (in view of the very low seasonal temperatures of waters in which lobsters live naturally) that at 1° C the pathogens persisted in the host in low numbers but with virulence unchanged, and they produced mortalities when the temperature increased. Experimentally infected lobsters were also sensitive to and died from rapid increases or decreases in environmental temperatures—although the temperature changes used in the experiments were probably greater than those that would normally be experienced in nature.

Findings in vivo were paralleled by in vitro results of growth of *Gaffkya* in lobster serum—with a more rapid increase to a peak of bacterial numbers with increasing temperature. The organism grew in culture at all the experimental temperatures (within a range of 1° to 20° C); at 1° C the bacterial growth curve was erratic—it decreased in numbers to the 30th day, then a log increase progressed to the 60th day, followed by a substantial decline. The important observation, of course, is that *G. homari* can survive within the range of environmental temperatures experienced by American lobsters and that the pathogen causes mortality more rapidly as temperature increases.

A concurrent physiological and biochemical study by Stewart, Arie, Zwicker, and Dingle (1969) and Stewart, Foley, and Ackman (1969), in which an attempt was made to define features of the infection that lead to death of lobsters, produced several interesting results. The pathogen lacked proteolytic, lipolytic, and fibrinolytic exoenzymes, suggesting that harmful effects are not caused by direct destruction of tissue. The authors observed that although in vitro growth of *G. homari* was limited by the carbohydrate level of the lobster serum medium used, presumably such a level would be maintained in vivo at the expense of other tissues. Drastic reductions in hepatopancreatic glycogen and hemolymph nonprotein nitrogen characterized later stages of the infection. No evidence of a toxin was detected, and the conclusion was that gaffkaemia is largely a wasting type of disease—that death from the disease was "a result of an unsuccessful competition on the part of the lobster for its own readily available storage material."

On the basis of experimental inoculations and subsequent mortalities, Bell and Hoskins (1966) suggested that *Gaffkya* might be pathogenic for the Dungeness crab, *Cancer magister*, and the shrimp *Pandalus platyceros* from the Pacific coast. That observation could be important in view of recent introductions of American lobsters (some possibly carrying *Gaffkya*) on the Canadian west coast. Other experimental studies (Cornick and Stewart, 1968b) indicated that the bacterium may also be pathogenic for east coast crabs (*Cancer irroratus*, *C. borealis*, and *Hyas coarctatus*). In vitro growth of the pathogen in crab sera was similar to that in lobster serum, suggesting susceptibility of the crabs. However, agglutinins for *G. homari*, which were demonstrated in the sera of one of the crab species (*C. irroratus*), might counteract the favorable bacterial growth in vivo and reduce the severity of infections in the crab. Cornick and Stewart extended their observations by inoculations of *C. irroratus* with suspensions of *G. homari*. After 49 days the surviving crabs (three) were found to be heavily infected (10^9 organisms·ml hemolymph). Passage through the crabs did not alter pathogenicity of *Gaffkya* to lobsters. A repetition of the crab inoculations with larger numbers of crabs provided some evidence of greater mortalities in experimental groups than in controls. Pathogenicity for rock crabs was less than for lobsters, as indicated by a mean time to death of 42 days in crabs, against only 18 days in lobsters. The authors mentioned the possible role of rock crabs as reservoirs of infection for lobsters, in view of reduced pathogenicity and prolonged mean time to death in crabs.

From the foregoing, it is apparent that experimental studies with *G. homari* have been numerous and varied and have provided significant insights about the internal defenses of Crustacea. Important areas for future study include determination of whether strains of the pathogen with different virulences exist, and determination of whether virulence may be increased by rapid passage through impounded lobster populations.

DISCUSSION

In the development of information and principles of invertebrate internal defenses, consistent and entirely natural attempts have been made to translate findings into the concepts and compartments constructed for the immune responses of vertebrates. The effort has led to some confusion of terminology and even to lack of agreement about definitions of immunity.

The concept of immunity in vertebrates has been admirably stated by Good and Papermaster (1964), who define immunity precisely and narrowly as "a biologic phenomenon embodying primary and secondary responses, with antibody synthesis and release, reactions of immediate and delayed allergy, and homograft immunity." They state: "To the time of writing [1964], adaptive immunologic responsiveness has not been demonstrated in the invertebrates." They then define adaptive immune responsiveness as "the ability to respond to antigenic material by production of specific combining substances, and to show an anamnestic response to these same antigens on subsequent exposure." Their continued exposition of immunity from the vertebrate point of view includes the following significant points:

1. "Adaptive immunity . . . is primarily a function achieving full expression late in phylogeny and ontogeny."
2. "The lymphoid cell family is the primary cellular basis for adaptive immune response in vertebrates . . . ."
3. "The possibility that another cell system may mimic adaptive immune responses in an invertebrate species cannot be excluded at this time."

A broader, more inclusive, concept of immunity has been suggested recently. If the broader definitions of terms proposed by several authors are accepted, the words "immunity" and "immune response," rather than careful circumlocutions, can be used with invertebrates. As an example, McKay and Jenkin (1969) stated that the Australian freshwater crayfish was capable of an "adaptive immune response." Such a capability is not possible within the confines of Good and Papermaster's definition of adaptive immunity as the production of specific immunoglobulins (a capacity which has been correlated with the occurrence of lymphoid tissue). Perhaps their definition is too restrictive and rigid, since a number of invertebrates do show responses that protect them from pathogens (hence they are adaptive).

Earlier definitions of antibodies and immunity allowed more latitude for inclusion of invertebrate responses. Cantacuzène (1923b), for example, considered as antibodies ". . . toute substance albuminoïde du plasma, douée ou non de spécificité, qui, se fixant sur l'antigène, modifie les relations de contact de ce dernier, soit avec les cellules, soit avec les autres constituants chimiques des humeurs."

McKay, Jenkins, and Rowley (1969) stated ". . . to allow comparisons to be made between invertebrate phyla and the vertebrates . . . the definitions of the immune response should be as broad as possible and emphasis placed on the functional aspects . . . ." These authors suggest that such a definition of the immune response might be "the ability of the animal to respond to a foreign particle (whether it be truly foreign or unwanted self) by the production of specific proteins capable of reacting with the inducer, and the resultant of this reaction leading to phagocytosis."

Cushing (1967), in an excellent summarization of invertebrate immune mechanisms, stated "There is a growing consensus of observations supporting the view that while vertebrates and invertebrates may share some basic immune competences such as 'innate immunities' and phagocytic cells, it is indeed only within the vertebrates that the full capacity of adaptive immunity exists." This statement seems reasonable, and fits the confines of Good and Papermaster's narrow definition of adaptive immunity, but is too negative if a broader perspective of immunity—such as that proposed by McKay, Jenkins, and Rowley—is adopted.

Probably greater emphasis should be placed on the *adaptive* aspects of invertebrate internal defense processes. Substantial numbers of studies have indicated the existence of adaptive responses to experimental inoculations of for-

eign protein. Whether the response is in the form of specific immunoglobulin seems less significant than the degree of protection afforded to the individual by the adaptive response. In the broadest sense, the replacement of a protective constituent of the hemolymph after its utilization in preventing infection could be considered adaptive. For example, the precipitating factor for foreign protein found by Stewart and Foley (1969) in lobster serum (which decreases following experimental inoculation of BSA and which is unreactive against injected lobster serum) would be adaptive.

Considering immune responses of vertebrates and invertebrates, Good and Papermaster stated that the presence in vertebrates of lymphoid tissue and cells constitutes a basic distinction. On this basis, as Chadwick (1967) has pointed out, "It is highly unlikely that insects [or Crustacea or other invertebrates] do produce mammalian type antibody, or that the mechanism of any acquired response to antigenic stimulus could be likened to responses in higher animals in terms of the production of specific antibody globulins." Analogous tissues and cells exist in a number of invertebrate groups, however, as do analogous humoral responses without the extreme specificities of vertebrate globulins. Chadwick (1967) also stated that the ". . . immune response in an insect is not the consequence of an antigen-antibody-globulin reaction but more likely the result of the production of some, as yet undefined, principle in insect hemolymph which may contribute to its resistance." The same statement might be made about other invertebrates in which an induced response has been demonstrated.

Although somewhat beyond the confines of the present consideration of internal defenses of Crustacea, it might be well to call attention to recent tissue transplantation work of Cooper (1968, 1969a, 1969b, 1969c, 1969d) with annelids, which indicates a high degree of specificity of response and which suggests some similarities to vertebrate tissue graft responses. Cooper's (1969d) concluding statement is significant: "Further clarification of anamnestic responses to tissue transplants would confirm our views that at least two of the parameters of adaptive immunity [in the vertebrate sense], namely specificity and memory, did not evolve exclusively with the lower vertebrates."

It is obvious that modification, redefinition, or replacement of some conventional immunological terminology—particularly toward broader definitions—is needed if the invertebrates are included in comparative immunology. If we remove "antibodies" from invertebrate terminology we must also remove "antigen," since antibody response is part of the definition of antigen. An effective substitute for "antigen" (as suggested for insects by Hinton) (Chadwick, 1967) would be "immunogen." Chadwick also suggested replacement of "antibody" with such terms as "natural bactericidal substance," "specific inducible substance," and others. Furthermore, as stated earlier, it must be made clear that when "lysins," "precipitins," "agglutinins," and other humoral factors of invertebrates are discussed, identification with vertebrate factors is not intended—the terms are used merely to indicate the kind of activity produced (i.e., "lytic substance or activity," "precipitating substance or activity," etc.) regardless of the physiological-biochemical mechanism(s) involved.

When suitably qualified the "safe" general terms, therefore, include "resistance," "immunity," "immune response"; terms that can have general applicability and utility, if accepted in a general sense, include "agglutinin," "lysin," "precipitin"; specific vertebrate terminology, not applicable to invertebrates includes "antibody," "antigen," and "serological."

Beyond the establishment of working definitions of immunity in invertebrates, it seems appropriate to list a number of generalizations that seem warranted by the admittedly narrow base of evidence now available. Obviously, any generalization about a group as evolutionarily diverse as the invertebrates—or for that matter even of the Crustacea—must be in the form of a tenuous and easily retractable hypothesis (which may at times border on speculation), and the following statements are offered with these qualifications:

1. Resistance in the vertebrates seems primarily related to production of immunoglobulins

which combine with foreign protein to enhance the phagocytic process. Although specific immunoglobulins have not yet been demonstrated in invertebrates, an analogous protein system, of lower specificity but with functions similar to vertebrate immunoglobulins, is suggested. Natural (and in some cases, partially specific) agglutinins are common in invertebrate body fluids, and their titers in some species may be increased by exposure to specific antigens.

2. Resistance in the vertebrates, and in some invertebrates also, includes the production of bactericidins, lysins, and agglutinins. The appearance of, or the increase in, titers of such factors in certain invertebrates following exposure to foreign proteins may account in part for increased resistance to certain pathogens (McKay and Jenkin, 1969; Bang, 1967b).

3. Many of the bactericidal, bacteriostatic, lytic, and agglutinating properties seem to be conferred on the hemolymph of invertebrates by release of materials from hemocytes. The substances so released often seem not only less specific in their action than vertebrate antibodies, but also the stimulation of release may be much less specific. For example, the release of a lysin in sipunculids for the ciliate *Anophrys* can be stimulated by inoculation of certain bacteria (Bang, 1967a), and the release of a hemolysin in *Maia squinado* for sheep red blood cells can be induced by injection of sipunculid coelomic fluid (Cantacuzène, 1920a, 1923b).

4. Immune response in invertebrates, as best exemplified in insects and crustaceans, is often rapid in onset and disappearance—usually a matter of a few days.

5. As has been observed by a number of authors (Feng and Stauber, 1968; Stewart, Cornick, and Zwicker, 1969), environmental temperature is a critical factor in the host-parasite relationships of invertebrates. Temperature has been found experimentally to exert a significant effect on the appearance, degree, and duration of resistance to infection in certain invertebrates (McKay and Jenkin, 1969), just as it does in poikilothermic vertebrates (Bisset, 1946, 1947a, 1947b, 1948a, 1948b). Temperature affects the rate of growth and reproduction of microorganisms, the rate of production of toxic metabolites, and the utilization of nutrient derived from the host. Temperature can also affect the rate of phagocytosis and the rate of production of humoral defenses against infection. Thus the progress of infection and the outcome of disease represents a composite of enhancement or inhibition partly mediated by temperature.

6. Endotoxin has been found (in the vertebrates) to increase the metabolic rate of phagocytes and stimulate phagocytosis (Jenkins and Palmer, 1960; Whitby et al., 1961). A similar effect may be produced by endotoxin in the invertebrates. Thus, exposure to gram-negative bacteria which are so abundant in the sea (or to their endotoxins) may increase the level of nonspecific resistance to other gram-negative organisms or their endotoxins. This "nonspecific immunity," which is also known in the vertebrates (Rowley, 1956; Landy and Pillemer, 1956), may be of great significance in the invertebrates—in fact, it may be the basic mechanism of internal resistance to bacterial pathogens in the invertebrates.

7. Handling and experimental procedures rapidly induce bacteremias in a number of invertebrates. Rabin (1965), for example, found that almost half of all American lobsters used in his studies had bacteremias upon arrival in the laboratory. Cornick and Stewart (1966) found that about 20% of a large sample of lobsters had bacteria in their hemolymph. Isolates were principally *Micrococcus*, *Pseudomonas*, *Brevibacterium*, and *Achromobacter*—bacteria commonly found in the marine environment, and apparently nonpathogenic for lobsters. The acts of capture, transport, and impoundment of these and other marine animals may produce stresses and physiological changes (or mechanical damage) that permit entry of microorganisms common in the surrounding sea water.

8. There is some limited evidence that the process of phagocytosis, fully elaborated in the Protozoa, is enhanced in the vertebrates by nonspecific humoral factors which sensitize, agglutinate, immobilize, or otherwise increase the susceptibility of proteins to phagocytosis by fixed and mobile phagocytic cells. Although the opsonizing substances of invertebrates have not

been adequately characterized and the mechanisms involved have not been adequately elucidated, it may be speculated that in the vertebrates a greater degree of specificity of humoral factors has been added to the nonspecific mechanisms found in the invertebrates.

The proper role of specific vertebrate antibody as a possible augmentation of evolutionarily older nonspecific internal defenses was alluded to by Miles (1962). He stated ". . . it is fairly clear that antibody *per se* has little effect on the viability or metabolism of microbes with which it combines. It is effective in defense either because it neutralizes toxins, or because it makes the microbe susceptible to non-specific defense factors like complement or the phagocyte . . . . We may then properly consider antibody as accessory to the more fundamental nonspecific defense mechanisms . . . ." It should be emphasized, however, that much remains to be learned about the nonspecific humoral factors of vertebrates, as well as invertebrates.

9. Brown (possibly chitinoid) bodies or cysts in gills characterize later stages of a number of crustacean diseases. The sequence of events, after invasion by microorganisms, may include action of toxic or inhibitory factors in hemolymph, accretion of moribund or dead invaders in gill lacunae, phagocytosis of dead organisms, and formation of nodules or cysts containing dead organisms, and gradual phagocytic destruction of necrotic material.

10. An important point, as Bang (1967b) mentioned, is that the probability of discovering internal defense mechanisms is greater when disease phenomena are studied under natural conditions. In experimental work, microorganisms pathogenic to marine invertebrates should constitute test organisms of choice; microorganisms found in the environment (and which may be facultatively pathogenic) should be next in order of preference; and microorganisms or proteins which the marine animal is unlikely to encounter seem least instructive. There are valid experimental reasons, of course—such as the ease of recognition of bacteriophages—that often lead to selection of test microorganisms other than pathogens or potential pathogens. Whether these unusual choices are effective antigens is obviously a most important consideration.

Another extremely pertinent observation made by Bang (1967b) was: "The limited amount of information [concerning immunological responses of invertebrates] is, I believe, due mainly to the limited number of studies, and not to any lack of imagination on the part of evolutionary forces in developing protective mechanisms."

11. One final and very significant thought was proposed by Stauber (1961): "That so few examples of acquired resistance are known among invertebrates may even be quite logical. Because of their relatively short generation times, their usual small size and often enormous reproductive capacities, subsequent epizootics would be much more likely to be circumvented by the appearance of resistant stocks through natural selection . . . . Even with very high mortality rates a residual stock of animals under favorable conditions later might repopulate an area . . . . If this reasoning is adequate to explain the lack of evidence for the occurrence of acquired resistance in most of the invertebrates, perhaps those invertebrates with a long life span, like *Limulus* should be investigated more fully, as likely hosts capable of demonstrating acquired resistance."

It is interesting to note that it is precisely those invertebrates with a long life span which have received increased attention during the past several years, and that a few indications of acquired resistance have been reported.

Information about the internal defenses of crustaceans and other invertebrates may be summarized as follows:

The weight of evidence indicates a major defensive role in invertebrates for phagocytosis, augmented by relatively nonspecific innate or acquired humoral factors. Preformed substances released into the hemolymph from granular hemocytes seem to play a major role in humoral defenses of Crustacea, and probably other invertebrates as well. Thus the body fluids of many invertebrates contain natural bactericidins, agglutinins, lysins, and occasionally precipitins. Some limited evidence for augmenta-

tion of such defenses by exposure to foreign antigen exists. This evidence is primarily in form of increased titers following experimental inoculation. Specific acquired antibodies (immunoglobulins) have not been demonstrated in invertebrates, but induced antibody-like activity has been demonstrated in a few species. The fundamental difference seems to be in degree of specificity of response, which is significantly higher in the vertebrates. It is obvious that the synergistic action of phagocytes and humoral factors in the invertebrates, as well as in the vertebrates, constitutes the significant defense perimeter—but the degree of specificity of the humoral components is lower in the invertebrates. The master internal defense plan seems to be: foreign protein + humoral factor = recognition of foreignness and phagocytosis.

Cooper (1969c), concluding a very thought-provoking paper, stated: "It seems reasonable to conclude that invertebrates do possess immune systems, although the nature of the mechanisms is decidedly unknown. Reactions may be as numerous as the varied taxonomic groups, as is true of most rigorously studied vertebrates. Invertebrate cellular immunity may be closer to vertebrate reactions and may represent the more primitive responses. In the absence of classic vertebrate-type immunoglobulin in invertebrates, a real dichotomy would be evident in the evolution of immune responses. On the other hand, immunoglobulin precursors may be present."

Certainly the next decade will prove to be an exciting one in the study of invertebrate internal defense systems. The components of classical vertebrate immunity—present as analogues in invertebrates, and probably varying widely among phyla—provide an excellent background against which new findings may be evaluated.

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CLEANING SYMBIOSIS AMONG CALIFORNIA INSHORE FISHES[1]

EDMUND S. HOBSON[2]

ABSTRACT

Cleaning symbiosis among shore fishes was studied during 1968 and 1969 in southern California, with work centered at La Jolla. Three species are habitual cleaners: the señorita, *Oxyjulis californica*; the sharpnose seaperch, *Phanerodon atripes*; and the kelp perch, *Brachyistius frenatus*.

Because of specific differences in habitat, there is little overlap in the cleaning areas of these three species. Except for juvenile sharpnose seaperch, cleaning is of secondary significance to these species, even though it may be of major significance to certain individuals. The tendency to clean varies between individuals. Principal prey of most members of these species are free-living organisms picked from a substrate and from midwater—a mode of feeding that favors adaptations suited to cleaning.

Because it is exceedingly abundant in a variety of habitats, the señorita is the predominant inshore cleaning fish in California. Certain aspects of its cleaning relate to the fact that only a few of the many señoritas present at a given time will clean, and that this activity is not centered around well-defined cleaning stations, as has been reported for certain cleaning fishes elsewhere. Probably because cleaners are difficult to recognize among the many señoritas that do not clean, other fishes generally do not attempt to initiate cleaning; rather, the activity is consistently initiated by the cleaner itself. An infested fish approached by a cleaner generally drifts into an unusual attitude that advertises the temporary existence of the transient cleaning station to other fish in need of service, and these converge on the cleaner. Although señoritas, as a group, clean a number of different fishes, a given individual tends to initiate cleaning with members of just one species.

The fishes cleaned most often are those which are most abundant and, at the same time, are most heavily infested with external parasites. The most numerous ectoparasites are caligid copepods, the most abundant and widespread of which is *Caligus hobsoni*. These particular parasites, along with gnathiid isopod larvae, are the major prey of the cleaning fishes. Cleaning is essentially limited to the external body surface; ectoparasites of the oral and branchial cavities are not ordinarily taken. Cleaning effectively reduces the number of parasites on fishes that are cleaned, and is an important activity for the organisms involved. However, there is no basis for the contention that many good fishing grounds in southern California exist because fishes have congregated in these locations for cleaning.

It has been suggested that many of the better inshore fishing spots are, in fact, cleaning stations (Limbaugh, 1961; Feder, 1966). The contention is that fishes congregate at these locations so that ectoparasites and other deleterious material can be removed from their bodies by resident cleaning organisms. Critics of this hypothesis might well suggest instead that cleaners simply are especially active where fishes are most abundant, or that the cleaners as well as those they clean occur at these locations for reasons that have nothing to do with cleaning. Regardless of which view is correct in a given situation, one having witnessed fishes crowded around a cleaner, vigorously soliciting its services, can only conclude that this activity is indeed important to the organisms involved.

Cleaning symbiosis has been widely described in the literature (Longley and Hildebrand, 1941; Eibl-Eibesfeldt, 1955; Limbaugh, 1955, 1961; Randall, 1958, 1962; and others) and was reviewed by Feder (1966). Youngbluth (1968) studied activity of the Hawaiian cleaning labrid *Labroides phthirophagus* in some detail, and Losey (1971) analyzed the communicative signals between this same species and the fishes that it cleans. But most other reports on cleaning have been simple treatments based largely on

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incidental observations. In this report, I describe cleaning symbiosis among inshore fishes of southern California and attempt to relate observed activity with the incidence of specific ectoparasites.

Conrad Limbaugh, Scripps Institution of Oceanography, was among the first to report cleaning symbiosis among California fishes. In a study of fishes of the kelp beds, Limbaugh (1955) described cleaning by the señorita, *Oxyjulis californica*, a fish of the family Labridae, and also by several seaperches of the family Embiotocidae: the kelp perch, *Brachyistius frenatus;* the black perch, *Embiotoca jacksoni;* and the pile perch, *Rhacochilus vacca*. Subsequent observers have described cleaning by the rainbow seaperch, *Hypsurus caryi* (Gotshall, 1967); the sharpnose seaperch, *Phanerodon atripes* (Clarke, Flechsig, and Grigg, 1967; Gotshall, 1967; Hobson, 1969a); and the blacksmith, *Chromis punctipinnis* (Turner, Ebert, and Given, 1969).

SPECIES STUDIED

Most of the cleaning observed during this study was performed by the señorita (Figure 1), which by virtue of its great abundance in a variety of habitats is the predominant cleaner inshore. The sharpnose seaperch (Figure 2) was frequently observed cleaning, but its activity is centered in deeper water. The kelp perch (Figure 3) may be an important cleaner in the canopy region of the kelp forests, where it concen-

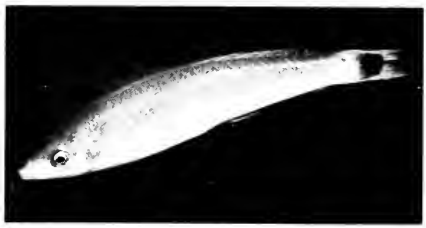

FIGURE 1.—Señorita.

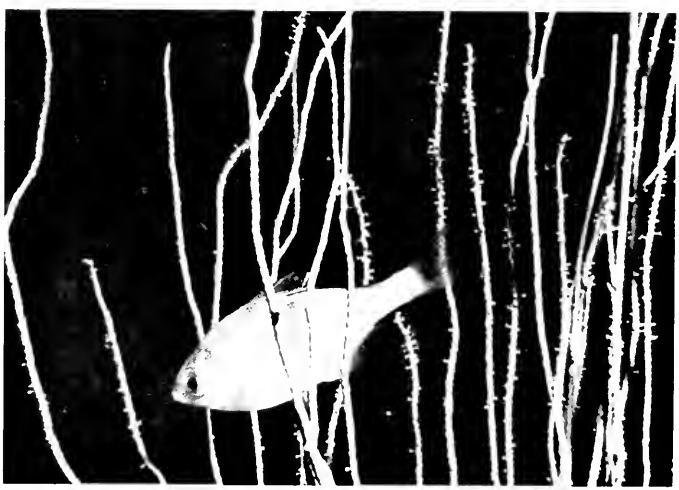

FIGURE 2.—Sharpnose seaperch among branches of a gorgonian.

trates, but this was not determined in this study because observations in the kelp-canopy habitat were infrequent. Nevertheless, observations were sufficient to recognize the kelp perch as a habitual cleaner. The only other fish seen cleaning was the white seaperch, *Phanerodon furcatus*, in which cleaning seemed to be only an occasional incidental activity.

METHODS

During 1968 and 1969, I spent more than 103 hr underwater directly observing cleaning and related activity in California inshore waters. Also contributing to the study are many incidental observations of cleaning made during other work with California fishes between 1961 and 1970.

Supplementing the observations, 421 specimens of 39 species were collected with spear. These represent most of the species common in the study area that exceed a length of 100 mm (all lengths of fishes in this report are standard length). The ectoparasites were collected from all specimens and will be reported in detail elsewhere in collaboration with R. F. Cressey, U.S. National Museum. These collections also provided the material for descriptions of 11 species of copepods formerly new to science (Cressey, 1969a, 1969b, 1970; J. Ho, California State College, Long Beach, unpublished manuscript). Additional undescribed species may occur among a number of copepods from these collections presently under study by Z. Kabata, Biological Station, Nanaimo, British Columbia.

In addition to a survey of the ectoparasites, gut contents of known cleaning species, including material from 53 señoritas, 29 sharpnose seaperch, and 3 kelp perch, were analyzed.

Many ectoparasites leave their host when it is in difficulty, and some fishes regurgitate their stomach contents under stress. To reduce this loss, all specimens were individually sealed in plastic bags immediately upon capture, and while still underwater.

To acquire detailed data on the cleaning interaction, a number of individuals of cleaning species were kept under surveillance for periods

FIGURE 3.—Kelp perch next to giant kelp.

up to 15 min, and a verbal account of their activity was recorded on tape. The attempted standard of 15 min could not be maintained for all these observations because sometimes contact with the fish being watched was lost as the fish swam among vegetation or other fishes. Individuals followed included known cleaners as well as others that had not shown evidence of cleaning. In monitoring the activity of known cleaners, a record was kept of the time during which they showed an apparent cleaning interest in other fish and also the number of cleaning bouts in which they became involved. A cleaning bout is defined here as any cleaning activity involving a discrete group of fishes, whether this group includes one cleaner attending a single fish, or several cleaners attending a cluster of 40 to 50 fish. On various occasions I also recorded the number of times that the cleaner actually "picked" at the body of another fish, and precisely at what point on the body this action was directed.

Study Areas

Observations of cleaning symbiosis were made at many locations throughout southern California, including the Channel Islands, and at the Coronado Islands, Mexico. However, most of the data were collected during concentrated work at La Jolla, Calif. Here, three study sites were established, each including an area of about 100 m^2, that lie on a line running northwest offshore from La Jolla Point. Moving away from the beach along this line, the first station lies in 3 to 10 m of water about 200 m offshore, the second is in 20 to 25 m of water about 700 m offshore, and the third in 30 to 35 m of water about 1000 m offshore. The sea floor at all three stations is rocky and irregular, with many crevices and caves. Algae are not conspicuous at the two deeper stations, which are similar, but the rocks support a heavy growth of gorgonians. On the other hand, the nearshore region of the 3- to 10-m station is richly carpeted with surfgrass, *Phyllospadix*, and other parts of the inshore station are forested by large kelps, particularly giant kelp, *Macrocystis*, and feather-boa kelp, *Egregia*. However, these large kelps are sparse here in comparison to some areas nearby to the south and elsewhere in California. Other details of the principal study area will be introduced as they become pertinent.

During all observation periods at the La Jolla stations a record was kept of water temperatures from surface to bottom, horizontal visibility, and surge conditions.

OBSERVATIONS

GENERAL ECOLOGY

Señorita

The señorita, which attains a length of about 250 mm, is one of the most abundant fishes in the inshore waters of southern California, including the Channel Islands. It occurs from the shoreline to depths exceeding 40 m and is recorded from central California south to central Baja California, Mexico (Roedel, 1953). An inhabitant of water over rocky substrates and among sea weeds, the señorita sometimes swims singly, but more often in groups of from a few to many hundreds of individuals. Like other labrids, it is strictly a diurnal fish, taking shelter under cover at night.

Food habits.—The señorita feeds on a variety of benthic organisms from the surface of both algae and rocks. It also feeds heavily in the midwaters, taking small organisms in the plankton, as well as forms that are attached to or encrusted on drifting algal fragments. All this feeding is accomplished in a characteristic picking manner, a mode of feeding well suited to its pointed snout and the several long, curved canine teeth that project forward at the front of each jaw.

To determine the food habits of this fish in the study area, 26 specimens, 110 to 195 mm long, were speared randomly from the population at large. None of these were cleaning when collected. Food items in their stomachs, ranked as percentage of each item in the entire sample, were as follows: bryozoans encrusted on algae, 43%; caprellid amphipods, 32%; fish eggs, 3%; gammarid amphipods, 2.5%; unidentified crustacean fragments, 4%; and pelecypod mollusks,

2.4%. A number of items each made up less than 1% of the sample, including crab fragments, gastropod mollusks, pycnogonids, and a gnathiid isopod larva. Unidentified material constituted 16% of the sample. The gnathiid larva, a single individual from one señorita, was the only evidence of ectroparasites among this material.

Limbaugh (1955) stated that señoritas are omnivorous carnivores which feed on almost any animal material. Quast (1968) concluded that the principal foods of the señorita are small gastropods and crustaceans associated with algae. Because he found no crabs or pistal shrimps in the diet, Quast suggested that bottom feeding is infrequent; however, having seen señoritas frequently picking on the bottom, I believe there must be some other reason why these prey are not taken more often. Size may be a factor, as crabs and pistal shrimps generally are larger and more heavily shelled than most prey of the señorita.

Movements.—Although individual señoritas may range widely over the bottom in a given locality, they seem to operate within a restricted range. Individual fish, when followed, always criss-cross back and forth within a defined area. Twelve individuals, selected randomly from the population at large and kept under surveillance for 11 to 15 min each, showed no evidence of cleaning.

Señoritas are most abundant at the 3- to 10-m station and become progressively fewer with increasing depth offshore. Nevertheless, even at the 30- to 35-m station the species was among the most numerous present. Fluctuations in numbers were often apparent with changes in water temperature. Some of the movement is vertical. When a layer of colder water moved in over the bottom—a frequent phenomenon at the 20- to 25-m station—señoritas were especially abundant up in the water column above the thermal interface. Seasonal and other longer term changes may induce inshore/offshore movements in certain members of the population. The numbers present fall off noticeably when temperatures drop much below 13° C, but at least some señoritas were present no matter what the conditions. These comments on temperature effects are based entirely on casual evaluations of relative abundance under varying conditions.

Sharpnose Seaperch

The sharpnose seaperch is not regarded as a common species (e.g., Limbaugh, 1955), but was relatively abundant during this study over rocky substrates below 20 m in the La Jolla area. It grows to over 200 mm long and is recorded from Bodega Bay, central California (Miller, Gotshall, and Nitsos, 1965), south to the San Benito Islands, Mexico (Roedel, 1953). Most of those observed during this study were juveniles less than about 125 mm long that swam singly or, more often, in small groups of less than 10 individuals. Adults were seen only occasionally but sometimes swam in larger aggregations. All activity observed in these fish occurred during daylight. After dark they hover above the substrate and are alert, but their activity at this time, if any occurs, was not determined.

Food habits.—This seaperch takes a variety of benthic organisms from the surface of rocks, algae, gorgonians, and other benthic substrates. Prey are taken off the bottom in a characteristic picking manner similar to that of the señorita. However, the seaperch's dentition would seem less specialized for picking than that of the señorita; its conic teeth are relatively short and straight, and those at the front of the jaws are not notably longer than those on the sides, nor do they project forward.

To investigate the food habits of this fish in the study area, 13 individuals, 76 to 170 mm long, were speared randomly from the population at large. None of these were cleaning when collected. Food items in their stomachs, ranked as percentage of each item in the entire sample, were as follows: caprellid amphipods, 56%; chitons, 9%; planktonic copepods, 9%; isopods, 8%; limpets, 2%; pelycypod mollusks, 1%; and sponges, 1%. Unidentified material made up 14% of the sample. There was no evidence of ectroparasites in this material. One individual had fed heavily and exclusively on planktonic copepods, showing that this fish is not

limited to benthic prey. I have found no references to food habits of this fish in the literature.

Movements.—On the basis of limited observations, these fish do not seem to move around in their habitat as much as señoritas do. Nevertheless, they do show marked inshore/offshore movements that may relate to changing water temperatures. Unlike the ubiquitous señorita, this fish occurred in limited numbers that allowed assessing relative abundance through actual counts. It was never seen at the 3- to 10-m station but was reasonably abundant (10-20 individuals were counted during 15-min periods) on all visits to the 30- to 35-m station. At the 20- to 25-m station its appearance was irregular and closely followed temperature fluctuations. Generally it was rare or absent at the 20- to 25-m station when bottom temperatures rose much above 13° C, and was present (a maximum of 10 was seen during a 20-min period) when the temperature dropped much below this level. As most of the individuals seen were juveniles, a seasonal factor independent of temperature was probably operating here. Nevertheless, short-term temperature changes over the critical range (approximately 12°-14° C at the 20- to 25-m station) were consistently accompanied by the presence or absence of this fish. I emphasize that these assessments of abundance are relative to the numbers of the species regularly present. The señorita was always more abundant than the seaperch at all stations and under all conditions. Thus whereas the seaperch was considered abundant during a period in which 15 individuals were seen, at no time did I find so few señoritas present at any of the three La Jolla stations.

Kelp Perch

The kelp perch was not abundant in the La Jolla study area, where it was seen only at the inshore station. Its distribution is essentially limited to the kelp beds, which were not well developed in the study area at the time of this work. Nevertheless, it is very numerous in California inshore waters that are heavily forested with kelp. Attaining a maximum length of about 150 mm, the kelp perch is recorded from Vancouver Island, Canada, south to central Baja California, Mexico (Roedel, 1953). The kelp perch occurs near the rocky bottom at the base of giant kelp, as well as adjacent to the rising kelp stipes, but is most abundant just under the kelp canopy, near the water's surface. Typically, this fish occurs in aggregations of a dozen or more, but larger individuals frequently are solitary, especially those near the rocky sea floor. Most of my observations of kelp perch were made outside the La Jolla study area, the majority around the Channel Islands.

Food habits.—This perch feeds in a picking manner, similar to that employed by the señorita and sharpnose seaperch. It preys on a variety of organisms from the surface of the surrounding kelp and also feeds extensively on material suspended in the current. Its pointed snout and small, upturned mouth, together with a number of relatively long, curved canine teeth that project forward at the front of each jaw, are well suited to its mode of feeding. The dentition of this fish is similar to that of the señorita, a fact also noted by Hubbs and Hubbs (1954). I did not sample kelp perch from the population at large for food-habit analysis; all those collected were from known cleaning stations. However, Limbaugh (1955) stated that they feed on small crustaceans, particularly those that occur on giant kelp. Quast (1968), who also reported a predominantly crustacean diet, with a preponderance of amphipods, noted that some mollusks and bryozoans are taken as well.

Movements.—Limited observations indicate that aggregations of kelp perch in the canopy, and close to large rocks, remain relatively stable. Several aggregations that were observed over 2 to 3 months did not change appreciably in location or in numbers of individuals. Data on this point are scanty, however.

At night they hover in the same areas in which they are active in daylight, but their activity at this time, if any occurs, was not determined.

CLEANING ACTIVITY OF THE SEÑORITA

Unlike some other cleaners (see Feder, 1966), señoritas do not establish well-defined stations at which they receive other fishes seeking to be cleaned. Rather, the señoritas, as they move over the local area, approach and clean fishes wherever they encounter them.

Despite their great abundance, only a small segment of the señorita population seems predisposed to clean at a given time. The cleaning habit is not limited to any particular stage in their life history: cleaning señoritas have included some of the smallest individuals seen (< 40 mm) as well as some of the largest (> 225 mm). In cleaning material from the bodies of fishes, señoritas employ the same picking technique they use to take small prey from a rock or algal substrate. This mode of feeding, along with their pointed snout and long, forward-projecting canine teeth, are well suited to the cleaning habit.

Individuals that clean are numerous where there are many resident fishes, especially of certain species (as discussed below), but I found no evidence that residents of other areas come to these locations to have parasites removed. Occasionally a migrating species, such as the California yellowtail, *Seriola dorsalis*, will pause to be cleaned while passing through areas where cleaners are active, but this is not the same as a resident of a particular area habitually swimming elsewhere to be cleaned and then returning to its home ground.

Fishes Cleaned by the Señorita

Casual observation alone show that some fish species are cleaned far more often than others, and that many species do not seem to interact with cleaners at all.

To obtain data on this point, a record was kept of the species seen being cleaned by señoritas during 62 observation periods (15 min to 2 hr long) from June 1968 to January 1969. During this period, 392 cleaning bouts were witnessed, 385 of which involved señoritas cleaning one or more individuals of a single species; in only seven instances were señoritas seen cleaning members of a mixed-species group. The tabulation of species cleaned (Table 1) does not include the mixed-species groups because in the mixed groups it was not determined whether representatives of all species present were actually cleaned. All seven mixed groups included halfmoons, *Medialuna californiensis*, and one or more fish of other species. In four of these, halfmoons were mixed with blacksmiths, in one they were mixed with opaleyes (*Girella nigricans*), in one with rubberlip perch (*Rhacochilus toxotes*), and in one with both rubberlip perch and pile perch. All of these were incidental observations. The compilation does not include data obtained on other occasions when the activity of individual cleaners was recorded for extended periods.

The data clearly indicate that blacksmiths, and to a lesser extent topsmelt (*Atherinops affinis*), predominate as recipients of the señorita's cleaning efforts in the areas where the observations were made. Table 1 is not a definitive list of species cleaned by the señorita; nevertheless, it is evident that many species which co-occur with the señorita are not cleaned. At other times, in addition to all species noted in Table 1, I have seen *Seriola dorsalis* and *Trachurus symmetricus* being cleaned. But the ratio of species listed here generally is consistent with observations made on other occasions and at many different locations.

TABLE 1.—Fishes observed being cleaned by señoritas during 62 observation periods between June 1968 and January 1969 at La Jolla, Calif. (exclusive of seven mixed-species groups).

Species	Number of cleaning bouts	Percent of total bouts observed
Blacksmith, *Chromis punctipinnis*	231	60
Topsmelt, *Atherinops affinis*	81	21
Garibaldi, *Hypsypops rubicunda*	22	6
Halfmoon, *Medialuna californiensis*	19	5
Señorita, *Oxyjulis californica*	10	3
Rubberlip perch, *Rhacochilus toxotes*	8	2
Opaleye, *Girella nigricans*	5	1
Kelpfish, *Heterostichus rostratus*	3	1
Black perch, *Embiotoca jacksoni*	1	1
Pile perch, *Rhacochilus vacca*	1	1
Sargo, *Anisotremus davidsoni*	1	1
Blue rockfish, *Sebastes mystinus*	1	1
Olive rockfish, *Sebastes serranoides*	1	1

Reports in the literature present a comparable picture. Most published accounts of cleaning by señoritas describe the way blacksmiths cluster around this cleaner to solicit its attentions (Limbaugh, 1955, 1961; Feder, 1966; and others). Limbaugh (1955) observed the following fish being cleaned by señoritas: *Myliobatis californica, Stereolepis gigas, Paralabrax clathratus, Trachurus symmetricus, Atherinops affinis, Anisotremus davidsoni, Hyperprosopon argenteum, Rhacochilus vacca, Chromis punctipinnis, Hypsypops rubicunda, Girella nigricans, Medialuna californiensis,* and *Mola mola.* Turner et al. (1969) observed the following fish being cleaned by señoritas: *Sebastes* spp., *Atherinops affinis, Atherinopsis californiensis, Trachurus symmetricus, Seriola dorsalis, Chromis punctipinnis,* and *Mola mola.* Neither of these reports gives data on the relative frequency with which these different species were cleaned, but it is significant that many of the same species consistently appear in the reports of independent observers, while at the same time many other species that frequent these waters in large numbers are not mentioned. No doubt many species not yet reported are occasionally cleaned by señoritas, but there seems little doubt that a certain few species, the blacksmith in particular, predominate in this activity.

Specific Cleaning Interactions

The fishes cleaned by the señorita vary markedly in their habits and habitat, as well as in their relative numbers. These fishes do not seek out cleaning at a "station" established by the señorita, but rather receive the señorita on their own grounds during the course of their regular activity. Cleaning interactions often proceed differently with one of these species than with another. Some of these variations in cleaning activity are characterized below.

Señorita-blacksmith interactions.—The blacksmith is one of the most abundant fish over rocky substrates in California inshore waters, where it swims in large stationary aggregations in midwater. It feeds largely on zooplankton (Quast, 1968) and attains a length of about 250 mm.

Generally the first sign of an interaction occurs when a señorita swims up alongside a blacksmith in midwater and closely inspects its body. The blacksmith may then immediately stop swimming and, holding its fins motionless and erect, drift into an awkward-appearing posture. Usually the blacksmith is head-down, but sometimes turns on its sides or is tail-down. On some occasions the blacksmith presents a particular part of its body to the inspecting señorita. The señorita swims about this fish, usually pausing briefly to pick at its body. Immediately following the first sign of this activity other blacksmiths converge on the spot, so that very quickly 10 or more crowd around the cleaner (Figure 4). The señorita soon leaves the original blacksmith and may then move on to one of the others. It may also swim slowly away, whereupon the group of blacksmiths follows along, each attempting to position itself in the señorita's path. Although the señorita shows progressively less interest in the blacksmiths, they continue to crowd in its way. Soon the señorita shows no further interest in cleaning, and all but a few blacksmiths leave the group. The remaining few doggedly continue attempting to present themselves to the now-unresponsive cleaner. Eventually, however, these last blacksmiths lose contact with the cleaner as it swims off among the kelp or the many other señoritas in the surrounding water. Once they have lost contact with the cleaner the blacksmiths do not attempt to solicit cleaning from any of the many other señoritas around them.

On only two occasions did I note blacksmiths soliciting cleaning from a señorita that did not seem to have made an initiating gesture. Once the blacksmiths were very small, about 40 mm long, and in the other observation, at a depth of 27 m, little cleaning had been seen and relatively few señoritas were present. However, in both instances the señoritas were known by me on the basis of earlier observations to be individuals that clean. It is possible that the fishes soliciting attention recognized these señoritas as cleaners through some cue not noted by me. Sometimes when a señorita incidentally passes close to a blacksmith, the blacksmith noticeably pauses in its swimming and looks as

FIGURE 4.—Señorita cleaning the caudal peduncle of one of a group of blacksmiths that hover to solicit service.

though it is beginning to assume a soliciting posture; however, when the señorita swims on past, the blacksmith immediately resumes its original activity. Occasionally members of other species were seen responding similarly to passing señoritas. In most observations of cleaning, my attention was drawn to activity already in progress, so that it was not possible to determine whether cleaner or client had initiated the activity.

Individual señoritas that cleaned blacksmiths during many short-term observations were not seen cleaning any other species. This same situation held true for three individuals, known to have been cleaning blacksmiths, whose activity was monitored in detail on tape for 15 min. When observed for extended periods, señoritas were found to become involved in a succession of separate cleaning bouts. This activity was not restricted to one location but continued at various points over a relatively wide area. Periodically they joined cleaning already underway, or initiated cleaning themselves at a number of different locations—always with blacksmiths. I have no explanation for the fact that a señorita which becomes unresponsive and leaves one group of blacksmiths that still vigorously solicits its service may soon initiate activity again with another blacksmith.

The three individuals whose cleaning activity was monitored for 15 min joined in a mean of 4 separate bouts (range 2-6). For a mean of 11 min of this time (range 6.75-13.25 min) they showed an apparent cleaning interest in blacksmiths, or were accompanied by blacksmiths with which they had earlier initiated a cleaning interaction. When not thus engaged with blacksmiths, they swam in midwater showing no apparent interest in the fishes around them but occasionally picked at drifting scraps of debris, usually algal fragments. During much of the time that they swam in consort with blacksmiths, they closely inspected these fish and actually picked at their bodies a mean of 26 times (range 14-33). Of these picks, 27% were made at the base of the blacksmith's anal fin, 25% on the

caudal peduncle or caudal fin, 22% at the base of the pectoral fin, 10% somewhere on the body exclusive of a fin-base or head, 8% on the head, 5% at the base of the pelvic fins, and 3% at the base of the dorsal fin.

Clearly, the bases of the fins receive most of the attention from the senoritas. These data are consistent with the many more general observations made on other occasions. At no time during this study were señoritas seen to clean within the oral or branchial cavities of blacksmiths: all cleaning was directed at the body surface.

Señorita-topsmelt interactions.—The topsmelt, which attains a length of about 200 mm, is abundant in many inshore regions of California coastal waters, but its distribution is more spotty than that of the ubiquitous blacksmith. Like the blacksmith, it feeds largely on zooplankton, which it takes while swimming in large schools at the water's surface. Quast (1968) noted the similarity in diet between topsmelt and blacksmiths, and while acknowledging that their feeding areas may overlap, he pointed out that topsmelt normally swim higher in the water column.

In the La Jolla study area, topsmelt are concentrated at the inshore station over extensive fields of surfgrass that grow in 3 to 5 m of water. They are never far from the substrate in this relatively shallow water, even though they swim in large schools at the water's surface. They are more abundant than blacksmiths in this area, and here they predominate in the senorita's cleaning activity.

The cleaning interaction proceeds in much the same way as it does with blacksmiths: the activity is initiated when a senorita swims up to an individual topsmelt and begins to inspect it closely. Immediately other topsmelt converge on this pair to place themselves in the señorita's path, thus soliciting its attention. When presenting themselves motionless before senoritas, topsmelt frequently hover tail-down, in contrast to the head down posture most often assumed by blacksmiths. I saw señoritas clean only the external body surfaces of topsmelt. In the relatively shallow water where most of this activity was observed, senoritas break off contact with a group of topsmelt more readily than they do with blacksmiths, as they need only swim down to the substrate below, where the topsmelt seem reluctant to follow.

These shallow areas are frequently swept by surge, and the load of drifting debris in midwater is frequently heavy. In this area cleaning señoritas frequently leave the groups of topsmelt they are attending to inspect an object drifting in the water nearby. Sometimes they take the object into their mouths, sometimes not. Often when taken it is quickly rejected.

Attempts at extended observations on individual senoritas that had been cleaning topsmelt were largely unsuccessful. Too often before the observation had progressed far the señoritas disappeared among the surfgrass or other vegetation carpeting the sea floor in this area. However, two individuals were followed for 10 min each, during which time one entered into four, the other two, separate cleaning bouts. Between cleaning bouts these two swam over a wide area alone in midwater, occasionally picking at drifting debris. On several occasions they picked at benthic algae. Neither individual showed cleaning interest in species other than topsmelt, which was consistent with observations of other señoritas that cleaned topsmelt.

Señorita-garibaldi interactions.—The garibaldi, which attains a length of about 250 mm, is a solitary, highly territorial fish that lives close to the substrate. Especially during the reproductive season, when the males aggressively guard their nests among the rocks, these bright orange pomacentrids normally drive away all other fish that come near. They feed on sessile benthic invertebrates and are abundant at the 3- to 10-m station.

Garibaldis frequently are cleaned by señoritas. Most of the garibaldis seen being cleaned were swimming a meter or so above the bottom; I did not observe cleaners active around the garibaldis guarding nests among the rocks. All of the garibaldis seen being cleaned were solitary, which reflects their territorial nature. The señorita swims up to a garibaldi and closely inspects its body, thus initiating the action. Usually the garibaldi hovers motionless in a normal hori-

zontal attitude, its fins sometimes erect. The señorita may pick at a few places on the garibaldi's body—most often around the caudal region—but usually its attentions are brief, and soon it swims away. With blacksmiths and topsmelt, each cleaning bout is prolonged by the many other individuals that join at the cleaning site to crowd in the señorita's path. Nothing of this sort happens with the solitary garibaldi, which usually makes no attempt to follow the señorita when it leaves, so that each cleaning bout is relatively brief. After leaving one garibaldi, however, often the señorita quickly approaches another. In agreement with their cleaning of blacksmiths and topsmelt, señoritas known to have cleaned garibaldis were subsequently seen cleaning only other members of that same species. This was true during several short-term observations, and also when one individual was followed for 15 min, and a record of its activity was taped; this particular señorita initiated cleaning activity with 26 different garibaldis during the observation period as it swam over an irregular course among the rocks in an area where blacksmiths, topsmelt, and other species also were present. Each cleaning bout lasted a mean of 10 sec (range 7-25 sec), totaling 4 min 15 sec of the 15-min period. In nine of these bouts, the señorita inspected the garibaldi but did not pick at its body. In the other 17 bouts, the señorita picked at the garibaldi's body a total of 42 times, or a mean of about 2.5 times per bout.

All cleaning of garibaldi that I observed was directed at the external body surface.

Señorita-halfmoon interactions.—Halfmoons, which may exceed a length of 250 mm, usually swim high in the water column, frequently in large aggregations, but just as often in small groups or as solitary individuals. They are often abundant among rising stands of giant kelp. Their omnivorous diet, which includes a variety of benthic algae, along with bryozoans, sponges, and crustaceans (Limbaugh, 1955; Quast, 1968), indicates bottom feeding; however, much of this material is taken in midwater as drifting debris.

Considering their large numbers in many southern California coastal areas, halfmoons are not particularly abundant in the principal study areas. Still, they were frequently seen being cleaned by señoritas during this study. When many halfmoons were present, cleaning by the señorita progressed much as described above for blacksmiths. Yet when just one halfmoon was present, a frequent occurrence, the cleaning bouts were brief like those described above for the garibaldi. At least one halfmoon was present in all the mixed-species groups that I recorded when collecting the data presented in Table 1. I saw señoritas clean only the external body surface of halfmoons.

One señorita, seen cleaning a halfmoon, was kept under surveillance for 12 min before contact was lost. As the observation period began, the señorita picked at the halfmoon once and then moved away, swimming slowly and alone, 2 or 3 m over the substrate. After an uneventful 3 min, the señorita approached a second halfmoon, which promptly hovered in a head-down attitude. For 15 sec the señorita closely inspected this halfmoon and picked at its body three times before swimming away. It then continued on alone for the remaining 8½ min that it was under observation, still swimming slowly over a wide semicircular course 2 or 3 m above the rocks. During this time it passed many different fish without showing interest, but it did not pass another halfmoon. It did pick at three different pieces of floating debris but rejected all three immediately.

Señorita-señorita interactions.—Señoritas themselves are cleaned by other members of their own species. Despite the large numbers of señoritas that usually are present, I saw no groups converging on cleaning individuals, as regularly occurs with blacksmiths, topsmelt, and other abundant species. In most of the señorita's intraspecific cleaning interactions, the cleaner attends just a single individual, which usually hovers motionless in a normal horizontal attitude, except that its fins are erected; sometimes the mouth is open wide and gill covers are distended, but I saw señoritas clean only the external body surface of these fish. There was no indication that señoritas which clean other señoritas also clean other species. I fol-

lowed one individual for 10 min after having seen it clean another señorita. After this initial activity, the individual under surveillance swam over a wide area, showing interest only in other señoritas, even though blacksmiths, topsmelt, and other species were present. Swimming alone, 2 or 3 m over the rocks, it would assume a position alongside another señorita and follow it for a short distance. Usually these other fish showed no interest, but some stopped swimming and erected their fins, whereupon the cleaner picked at their bodies—usually once, but occasionally several times. Between cleaning encounters this señorita passed through a school of very small (< 40 mm) blacksmiths, several of which hovered head-down in its path; however, the cleaner showed no interest in these fish. On two occasions it picked at a piece of drifting debris.

Señorita-kelpfish interactions.—At least one species regularly initiates cleaning bouts with señoritas. Earlier (Hobson, 1965a) I reported observing a kelpfish, *Heterostichus rostratus*, repeatedly soliciting cleaning from unresponsive señoritas. The kelpfish was concealed among benthic algae, which is the typical habitat of this fish. But each time a señorita approached in the water overhead, the kelpfish rose up into the señorita's path, where it hovered motionless, fins erect (Figure 5). A succession of señoritas passed by without responding to the kelpfish, and each time the kelpfish returned to the cover below, where it waited until the next approach. Finally a passing señorita paused briefly and picked at the kelpfish's side before continuing on its way. After this brief encounter, the kelpfish did not rise from concealment again even though several more señoritas subsequently passed overhead.

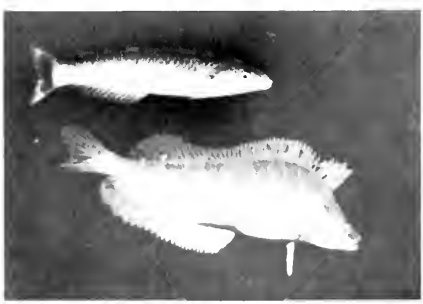

FIGURE 5.—Kelpfish hovering in midwater, fins erect, to solicit cleaning from a passing señorita.

During the present study this sequence of events involving kelpfish and señoritas was witnessed several times at a variety of locations; indeed, every instance of a kelpfish being cleaned followed this pattern—obviously it is a regular pattern in the behavior of the species.

Other interactions—Observations are too few to recognize distinctive aspects in cleaning interactions involving the many other species that occasionally are cleaned by señoritas. Usually such activity is noted simply as occasional sightings of small clusters of fish, or individual fish, hovering before a señorita. In all such encounters, however, only the external body surface was cleaned.

Notes follow regarding two other species that are cleaned by señoritas.

A single señorita was observed cleaning a pile perch, after which its activity was noted for 15 min. Pile perch are not abundant where these observations were made, and after leaving the first individual, the señorita swam alone in midwater for 14 min. It moved over a wide semicircular course during this time and showed no interest in any of the many fish that it passed, although none were pile perch. It did pick at three small items drifting in midwater. After 14 min it made an abrupt course change and, with slightly accelerated swimming, went directly to a solitary pile perch that was in midwater about 10 m away. The señorita swam about the pile perch, which now hovered head-down, but after a close inspection lasting about 10 sec it moved on without picking at the pile perch's body.

Many of the fishes cleaned by señoritas occasionally start, as if nipped too vigorously. Sometimes such fish dart away, thus terminating the cleaning. Other times they actively turn on the

señorita and drive it away. The rubberlip perch was noted taking the latter course of action perhaps more often than the other species, even considering the relatively few times it was observed being cleaned.

Although the kelpfish is the only species that was observed consistently initiating cleaning from señoritas, individuals of several species do so in at least one situation. This occurs where exceptionally large concentrations of señoritas, sometimes thousands, swim above the rocks. At various times, garibaldis, pile perch, rubberlip perch, olive rockfish, and others were observed hovering in the soliciting posture amid these concentrations (Figure 6) until one of the señoritas approached and cleaned them.

Material Removed from Other Fishes by the Señorita

Food habits of cleaning señoritas.—An obvious question is: What do señoritas remove from the bodies of fishes they clean? Limbaugh (1955) stated that señoritas remove bacteria, parasitic copepods, and isopods. He was not more specific than this, nor did he present data. Various other cleaners reportedly take not only ectoparasites but also diseased and necrotic tissue (Feder, 1966, and others.)

To determine just what it is that señoritas remove from the bodies of other fishes, I examined the gut contents of 27 specimens, 111 to 175 mm long, that were speared while they were cleaning other fishes. Food items in their guts, ranked as percentage of each item in the entire sample, were as follows: caligid copepods, 39%; gnathiid isopod larvae, 12%; algae with encrusting bryozoans, 10%; caprellid amphipods, 5%; fish scales, 4%; and fragments of nonparasitic crustaceans, 4%. Unidentified material made up 26% of the sample. Of the 27 specimens, ectoparasites occurred among the gut contents of all but two. In most, the ectoparasites predominated. Even though the data are convincing, they do not fully reflect the extent to which cleaning obviously dominated the activity of these particular fish for at least several hours leading up to their capture. This is because the

FIGURE 6.—Garibaldi hovering amid a large assemblage of señoritas.

parasites are so small (1-3 mm long) in comparison with the size of other food items. For example, 471 gnathiid isopod larvae were present in one señorita gut, but being so small they constituted only 35% of the material. On the other hand, a few algal fragments, with encrusting bryozoans, made up 40% of the material in this same specimen. However, only seven individuals contained ectoparasites alone, as compared with 17 that contained both parasites and free-living prey. In all but one of these, the two classes of material were sharply divided in the gut, usually with the free-living material posteriorly in a more advanced stage of digestion. Although most of the ectoparasites in the gut had undergone extensive damage and would not, by themselves, have been identifiable to species, this material usually graded gradually to freshly ingested specimens that were readily identified. This fact, coupled with the circumstance that individual señoritas tend to stay pretty much with a single type of food organism during a given period, greatly aided the task of analyzing this material.

Ectoparasites on the fishes.—To assess the significance of cleaning in removing ectoparasites, one must know what parasites occur on the fishes, as well as the extent of the infestation. Thus the survey of ectoparasites done in conjunction with this work included essentially every fish species exceeding 100 mm long regularly present in the La Jolla study area, as well as every species that was seen being cleaned there. Ectoparasites infesting these fishes include 33 species of copepods, one species of brachiuran, two species of isopods, one species of leech, and one species of monogenetic trematode. Following is a brief summary of the information being compiled on these parasites in collaboration with R. F. Cressey.

Copepods are the predominant ectoparasites on fishes in this area. The 33 species represent seven families: Bomolochidae (6 species), Caligidae (13 species), Dichelesthiidae (2 species), Lernaeidae (1 species), Chondracanthidae (5 species), and Lerneopodidae (6 species). The one species of the closely related brachiurans is a member of the family Argulidae. The bomolochids, which were found on 12 species of the fishes sampled, are mobile forms about 2 mm long (all lengths of parasites here and below do not include egg cases) that occurred mostly on the gills of their hosts. The caligids, which infested 29 species of the fishes, are mobile forms, 2 to 4 mm long, that occurred mostly on the external body surface of their hosts, although two species were found only in the oral cavity. The dichelesthiids are highly modified forms about 2 mm long that were attached to the gills of two species. The lernaeid is a highly modified form about 5 mm long that was attached to the fins of 12 species. The chondracanthids, which infested five species, are highly modified forms, 3 or 4 mm long, that lived attached in the branchial chamber, including the gills, of their hosts. The lerneopodids, infesting eight of the fish species, are highly modified forms, 2 to 5 mm long, mostly living attached in the branchial and oral cavities, although one individual fish carried several attached to its dorsal fin. Finally, the argulid is a mobile form about 2 mm long that was found on the outer body surface of one species of fish. The fish species hosting representatives of the different copepod and brachiuran families are listed in Table 2, and examples of the six copepod families are illustrated in Figure 7.

Thus a variety of ectoparasitic copepods occur on the fishes, but only caligids were found among the gut contents of the cleaners. Furthermore, although 13 species of caligids (five species of the genus *Caligus* and eight species of the genus *Lepeophtheirus*) occur on fishes in this area, only a relatively few of these are significant as prey of the cleaners, as noted below.

Of the two isopods, one, *Livoneca vulgaris*, a large parasite, about 20 mm long, was found in the branchial chamber of just one species of fish and was not found to be prey of the cleaners. On the other hand, the highly mobile gnathiid larvae (Figure 8), which are about 2 mm long, are a major prey of the cleaners. Only one form of gnathiid was readily recognized, but more than one species may occur among this material. Parasites of the body surface of fishes, the gnathiid larvae were taken on 11 of the fish species sampled, but I suspect that they are actually more widespread and abundant than these data

TABLE 2.—The types of ectoparasites and the fishes they infest, based on a survey of the fishes in the study area. Where more than one species of parasites is included under a heading, the number in parentheses following the name of each fish thereunder indicates how many different species of that type are represented on that fish. For all fish species listed, the number of infested individuals is shown over the number of individuals examined, followed by the range in numbers of individual parasites of that type which were taken from that species of fish.

COPEPODS

ŌMOLOCHIDAE (6 species of 3 genera)

Fishes infested with members of this family:

Paralabrax clathratus (1) 2/8 1-2
P. nebulifer (2) 5/11 1-20
Phanerodon atripes (1) 1/13 1
Rhacochilus vacca (1) 3/15 1
Micrometrus minimus (1) 1/7 1
Hypsypops rubicunda (1) 1/20 1
Scorpaena guttata (1) 6/14 2-56
Sebastes mystinus (1) 1/9 1
S. serranoides (1) 2/11 1-8
Oxylebius pictus (1) 1/8 1
Atherinops affinis (1) 2/13 1-4
Pleuronichthys coenosus (1) 6/10 1-11

CALIGIDAE (13 species of 2 genera)

Fishes infested with members of this family:

Paralabrax clathratus (2) 4/8 1-5
P. nebulifer (3) 4/11 5-27
Caulolatilus princeps (1) 1/4 2
Anisotremus davidsoni (1) 1/8 1
Chelotrema saturnum (1) 2/16 1
Medialuna californiensis (2) 12/13 1-75
Girella nigricans (3) 1/10 1-14
Embiotoca jacksoni (2) 2/15 1-2
Phanerodon atripes (1) 5/13 1-5
Rhacochilus toxotes (3) 10/10 1-10
R. vacca (2) 4/15 1-11
Chromis punctipinnis (1) 10/10 2-39
Hypsypops rubicunda (2) 19/20 1-144
Pimelometopon pulchrum (3) 13/14 1-70
Oxyjulis californica (3) 13/38 1-59
Scorpaena guttata (1) 7/14 1-14
Sebastes atrovirens (3) 6/16 1
S. carnatus (2) 3/11 1
S. chrysomelas (1) 1/7 1
S. constellatus (1) 2/2 1-5
S. miniatus (1) 1/5 2
S. mystinus (3) 5/9 1-5
S. paucispinis (1) 3/3 1
S. serranoides (3) 8/11 1-10
S. serriceps (2) 15/15 1-25
Scorpaenichthys marmoratus (2) 8/10 1-42
Heterostichus rostratus (1) 2/13 1-2

COPEPODS—Cont.

CALIGIDAE—Cont.

Atherinops affinis (1) 13/13 1-23
Pleuronichthys coenosus (1) 5/10 1-5

DICHELESTHIIDAE (2 species of 1 genus)

Fishes infested with parasites of this family:

Gymnothorax mordax (1) 1/1 9
Paralabrax nebulifer (1) 5/11 2-70

LERNAEIDAE (1 species)

Fishes infested by this parasite:

Trachurus symmetricus (1) 1/7: 1
Anisotremus davidsoni (1) 1/8. 1
Chelotrema saturnum (1) 2/16 1-2
Medialuna californiensis (1) 5/13 2-7
Brachyistius frenatus (1) 1/5 1
Embiotoca jacksoni (1) 2/15 1-2
Hypsurus caryi (1) 2/11: 1-2
Phanerodon furcatus (1) 3/12 1-3
Rhacochilus toxotes (1) 1/10 1
R. vacca (1) 2/15 1
Micrometrus minimus (1) 5/7 1-6
Atherinops affinis (1) 2/13 1-4

CHONDRACANTHIDAE (5 species of 5 genera)

Fishes infested with parasites of this family:

Oxyjulis californica (1) 13/38 1-4
Scorpaena guttata (1) 2/14 1-5
Scorpaenichthys marmoratus (1) 4/10 1-4
Heterostichus rostratus (1) 7/13 1-7
Pleuronichthys coenosus (1) 7/10: 1-47

LERNEOPODIDAE (6 species of 4 genera)

Fishes infested by parasites of this family:

Chelotrema saturnum (1) 1/16 1
Girella nigricans (1) 1/10. 1
Phanerodon atripes (1) 5/13 1-4
Rhacochilus vacca (1) 1/15 1
Chromis punctipinnis (1) 1/10. 1
Sebastes atrovirens (1) 1/16. 4
S. constellatus (1) 1/2 1
S. miniatus (1) 5/5: 2-8

ARGULIDAE (1 species)

Fish infested with this parasite:

Atherinopsis californiensis 1/1: 2

ISOPODS

CYMOTHOIDAE (1 species)

Fish infested with this parasite:

Sebastes mystinus 1/9 1

GNATHIID LARVAE (number of species not determined)

Fishes infested with these parasites:

Chromis punctipinnis 1/10 1
Hypsypops rubicunda 4/20 1-8
Pimelometopon pulchrum 3/14 1-4
Sebastes atrovirens 4/16: 1-20
S. carnatus 3/11: 1
S. chrysomelas 1/11: 2
S. constellatus 1/2: 1
S. serranoides 1/11: 1
S. serriceps 3/15. 1-5
Oxylebius pictus 3/8 1-3
Scorpaenichthys marmoratus 3/10. 2-5

MONOGENETIC TREMATODE (1 species)

Fishes infested with this parasite:

Medialuna californiensis 6/13: 2-16
Girella nigricans 4/10 1-4
Rhacochilus toxotes 2/10 1
R. vacca 2/15 1-18
Hypsypops rubicunda 1/20: 1
Pimelometopon pulchrum 10/14 1-26
Scorpaena guttata 2/14 5-8
Sebastes atrovirens 7/16 1
S. constellatus 1/2. 1
S. miniatus 2/5 1-2
S. serranoides 3/11. 1-26
S. serriceps 2/15 1-3
Heterostichus rostratus 6/13 1-7

LEECH (1 species)

Fishes infested with this parasite:

Hypsypops rubicunda 2/20 1
Sebastes serranoides 1/11. 4
Heterostichus rostratus 2/13. 1-2

FISHES ON WHICH NO PARASITES WERE FOUND:

Xenistius californiensis 0/1
Halichoeres semicinctus 0/10
Coryphopterus nicholsi 0/5

indicate. They are the most mobile of the parasites, and probably many escaped when their host fish was collected. The monogenetic trematode occurred on the outer body surface of 13 species of fishes, and the leech occurred similarly on 3 species. However, neither was found to be taken by the cleaners. The fish species hosting the various isopods and also the trematode and leech are listed in Table 2. Listed also are the three fish-species on which no ectoparasites were found.

The above summary of the survey results gives a general picture of the ectoparasites infesting the fishes that co-occur with the cleaners and might be regarded as a list of the potential prey of the cleaning fishes. The following material considers the parasites that actually are known to be prey.

Ectoparasites in the diet of cleaners relative to ectoparasites on fishes that are cleaned.—Many of the ectoparasites listed above infest

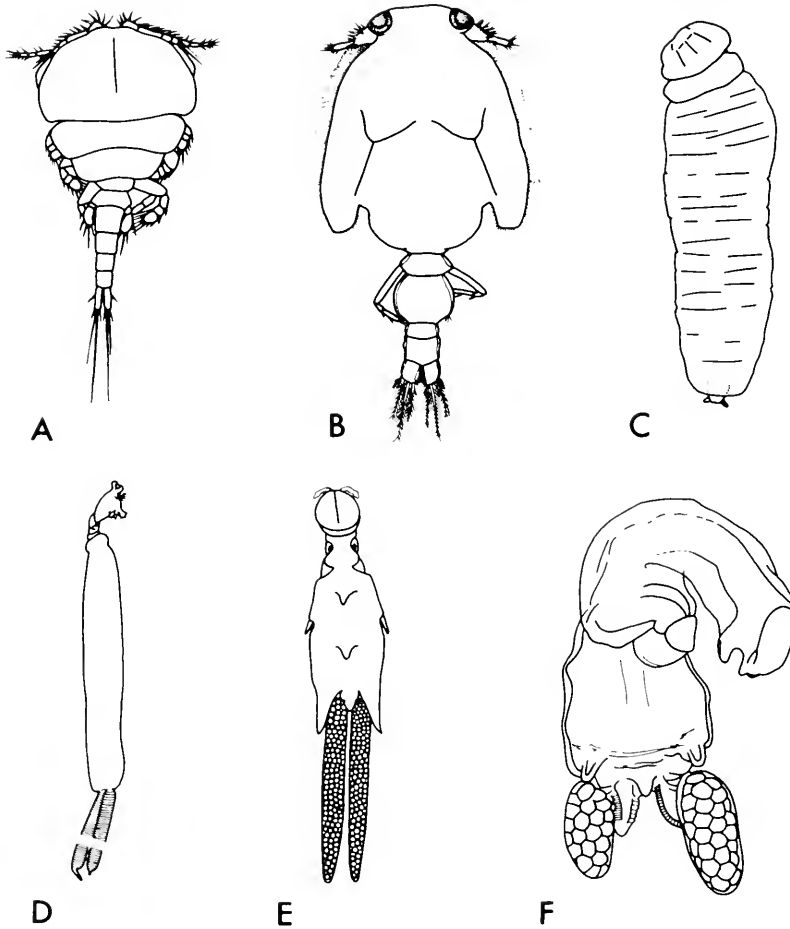

FIGURE 7.—Representatives of the families of ectoparasitic copepods found to infest fishes in the La Jolla area.

A. Bomolochidae (*Bomolochus longicaudus*, female, after Cressey, 1969b);
B. Caligidae (*Caligus kabsoni*, male, after Cressey, 1969a);
C. Dichelesthiidae (*Hatschekia pacifica*, female, after Cressey, 1970);
D. Lernaeidae (*Peniculus fissipes*, female, after Wilson, 1917);
E. Chondracanthidae (*Chondracanthus gracilis*, female, modified after Wilson, 1935);
F. Lerneopodidae (*Epibranchiella septicauda*, female, after Shiino, 1956).

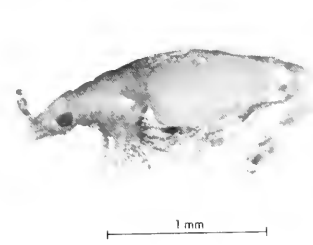

FIGURE 8.—Gnathiid larva from the body surface of the black-and-yellow rockfish, *Sebastes chrysomelas*.

fishes that rarely or never interact with cleaners. In considering the ectoparasites found in the gut of particular señoritas, it would be most meaningful to do so in regard to the ectoparasites known to be hosted by the species of fishes that these particular señoritas were cleaning when collected. Of the 27 cleaning señoritas taken for the gut-content analysis, 15 (56%) were cleaning blacksmiths, 8 (30%) were cleaning topsmelt, 2 (7%) were cleaning garibaldis, and 2 (7%) were cleaning halfmoons. Thus the selection closely parallels the relative frequency with which señoritas were observed cleaning these same species (Table 1) and is a good sample of the fishes that are cleaned by señoritas.

Three species of ectoparasites were collected from 10 blacksmiths, 141 to 199 mm long. Each of these blacksmiths carried from 2 to 39 individuals of the copepod *Caligus hobsoni* on their body surface. One specimen also carried a single gnathiid isopod larva on its body surface, and another the copepod *Clavellopsis flexicurvica* on a gill arch. All 15 señoritas that were collected as they cleaned blacksmiths contained either *Caligus hobsoni* or gnathiid larvae, but no other ectoparasites: one contained gnathiids alone, seven contained *C. hobsoni* alone, and seven contained both gnathiids and *C. hobsoni*. Up to 256 individuals of *C. hobsoni* and up to 263 gnathiid larvae were counted from among the stomach contents of individual señoritas that had been cleaning blacksmiths.

Three species of ectoparasites were collected from 13 topsmelt, 122 to 212 mm long. These topsmelt each carried from 1 to 23 specimens of the copepod *Caligus serratus* on their body surface. Two topsmelt also carried the copepod *Parabomolochus constrictus* on their gills, a single parasite on one, four on the other. Two topsmelt also carried the copepod *Penieulus fissipes* embedded in their fins. Six of the eight señoritas that had been cleaning topsmelt when collected had ectoparasites among their gut contents. Five contained only *Caligus serratus*—as many as 73 in each fish. One other contained only 10 gnathiid larvae, a parasite that was not seen on the topsmelt themselves; however, as noted above, I suspect that this parasite is more widespread than our survey data indicate.

Six species of ectoparasites were collected from 20 garibaldis, 184 to 240 mm long. Nineteen garibaldis each carried 1 to 144 *Caligus hobsoni* on their body surface. Thirteen each carried 1 to 4 individuals of an unidentified species of *Lepeophtheirus* on their body surface, and one carried a single *Bomolochus ardeole* in its branchial cavity. In addition, four carried 1 to 8 gnathiid isopod larvae, two carried a single leech, and one carried a single monogenetic trematode, all on their body surface. The two señoritas that were collected as they cleaned garibaldis had preyed mostly on gnathiid larvae, with each containing over 400 of these parasites. In addition, one had consumed six *Caligus hobsoni*, and the other had taken five *Lepeophtheirus* sp.

Four species of ectoparasites were collected from 13 halfmoons, 166 to 295 mm long. Twelve of the 13 halfmoons each carried 1 to 75 *Caligus hobsoni* on their body surface. Each of two also carried a single *Lepeophtheirus* sp. on its body surface, and each of six carried 2 to 7 *Penieulus fissipes* embedded in its fins. In addition, each of six carried from 2 to 16 monogenetic trematodes on its body surface. Of the two señoritas collected as they cleaned halfmoons, each contained only *Caligus hobsoni* in its gut contents, one a single specimen and the other eight.

Significantly, with the exception of the gnathiid larvae in a cleaner of topsmelt, as discussed above, no parasite was found in the cleaner's gut contents that did not occur on the species of fish that was being cleaned by the cleaner

when it was collected. This fact further supports the contention that cleaning tends to be species-specific for a given señorita.

The data clearly show that the parasites most frequently taken by señoritas are certain mobile forms that occur on the body surface of their host. It may be that other parasites on the external body surface are not taken. No leeches or trematodes were found among gut contents, even though these forms are abundant on the garibaldi and halfmoon. Also, the gut contents did not show evidence of the lernaeid *Peniculus fissipes*, an immobile form which partially embeds itself in the skin of its hosts—mostly on the fins. This parasite occurs on topsmelt, garibaldis, and halfmoons among those known to be cleaned by señoritas. However, negative evidence based on the meager gut-content data are weak, especially as the cleaning labrid *Labroides phthirophagus* in Hawaii, which feeds mostly on caligoid copepods, frequently takes lernaeids (Randall, 1958; Youngbluth, 1968). I would expect additional study to show that cleaning señoritas at least occasionally take *P. fissipes*. Nevertheless, several abundant fishes infested by *P. fissipes*, but not found to carry caligids, gnathiids, or other mobile external forms, were not seen being cleaned. For example, the white seaperch is one of the most abundant species at the 3- to 10-m station off La Jolla and yet was never seen being cleaned. Twelve specimens of this fish were examined, and the only ectoparasites found were one to four *P. fissipes* on three individuals. Similarly, the only parasite found on 11 rainbow seaperch, an abundant species in the study areas that was not seen being cleaned, was a single *P. fissipes* on one individual and two on another.

However, not all fishes whose external body surfaces are heavily infested by mobile forms were observed being cleaned. The sheephead, *Pimelometopon pulchrum*, is a case in point. *Caligus hobsoni* occurs on this fish, but only infrequently—a single specimen of this copepod was taken from each of 2 of the 11 sheepheads that were examined. However, the sheephead is heavily infested by two species of *Lepeophtheirus*, a genus of copepods that is closely related to *Caligus*. Up to 70 *L. parrus* were taken from the body surface of a single sheephead, and this fish has not yet been seen being cleaned. Furthermore, up to 4 gnathiid larvae, which cleaners take from other fish, were found on 3 of the sheephead. Similarly, the treefish, *Sebastes serriceps*, which is heavily infested with caligids, has not yet been seen being cleaned. The treefish is not known to carry *C. hobsoni*, but 13 of 15 specimens examined carried up to 12 *Lepeophtheirus longipes* on their body surface, and 3 carried up to 5 gnathiid larvae. The significance of these exceptions to what seems a valid generalization has not been determined. Perhaps it is significant that these two species of fish are not heavily infested by copepods of the genus *Caligus*, as are the more frequently cleaned fishes.

The many parasites that infest the oral and branchial cavities might seem to be potential prey for cleaners, but I found no evidence that these parasites are taken by señoritas.

The principal ectoparasites on the body surface of the two most frequently cleaned fishes, the blacksmith and the topsmelt, are the copepods *Caligus hobsoni* and *C. serratus*, respectively, which are very similar to one another morphologically. With just one exception among the fishes surveyed (discussed below), *C. serratus* seems to be restricted to topsmelt. On the other hand, *C. hobsoni* occurs on a wide variety of species and is also the principal form on garibaldis and halfmoons. Interestingly, a list of the fishes hosting this parasite, ranked by incidence (Table 3), looks much like the ranking of fishes that were observed being cleaned by the señorita (Table 1).

The importance of cleaning in reducing the incidence of ectoparasites on fishes.—Certainly cleaners remove many ectoparasites from the bodies of certain fishes—the numbers in their diet attest to this fact. But does cleaning in fact appreciably reduce the level of infestation on these fishes, or do other parasites quickly replace those that are removed by the cleaners? Although this question is difficult to answer, some insight is provided by observations on the garibaldi. When guarding eggs on their nests during the reproductive season, male garibaldis be-

come especially intolerant of the presence of other fish species. Clarke (1970) recorded the number of times garibaldis, in defence of their territory, attacked fish of various other species at different times of the year. He found that when males were guarding eggs their attacks on señoritas increased elevenfold. Not surprisingly, I saw no cleaning of garibaldis that were guarding eggs. At other times of the year male garibaldis do not guard their territory as vigorously against members of other species and are frequently seen being cleaned. A series of these males were collected both in and out of the reproductive season, and the numbers of ectoparasites they carried were assessed. Seven individuals (mean length 228 mm) sampled as they guarded their eggs carried a mean of 67 *Caligus hobsoni* (range 20-144), 2.5 *Lepeophtheirus* sp., 1.4 gnathiid isopod larvae, and 0.2 monogenetic trematodes. These counts contrast strikingly with those from six males (mean length 219 mm) sampled outside the reproductive season, which carried a mean of only 4.8 *C. hobsoni* (range 0-13), 1 *Lepeophtheirus* sp., 0.8 gnathiid larvae, and no monogenetic trematodes. These findings suggest that males which are guarding eggs become heavily infested with *C. hobsoni* when they do not allow cleaners to approach them, a conclusion strengthened by the fact that over this same period the relative numbers of this same parasite were not noted to change on other infested fishes. The samples included too few of the other parasites to make a meaningful comparison. It remains a question why *Lepeophtheirus* sp. and the gnathiid larvae did not show a pattern of occurrence similar to that of *C. hobsoni*, as both of these parasites are known to be prey of the cleaners. In any event, these data add to the evidence which indicates that *C. hobsoni* is the primary prey of cleaning señoritas in the study areas.

Ectoparasites on Señoritas

Señoritas that were closely observed as they cleaned other fishes often were noted to have caligid copepods on their bodies. One señorita, about 120 mm long, was host to an estimated 100 of these parasites concentrated especially along the dorsal-fin base. These observations were significant because during the survey for ectoparasites, most señoritas taken from the population at large were free of external forms, although many carried a chondracanthid copepod on their gills.

Twenty señoritas, 102 to 190 mm long, were sampled from among those giving no indication of being cleaners. Eight of these carried 1 or more of the chondracanthids on their gills, but only 2, or 10%, had parasites on their external body surfaces: one of these carried 10 specimens of *Caligus hobsoni* and 1 specimen of

TABLE 3.—Hosts of *Caligus hobsoni*.

Species	Specimens examined	Specimens hosting *C. hobsoni*	Number of *C. hobsoni* on each infested fish mean (range)	Percent occurrence
Blacksmith, *Chromis punctipinnis*	10	10	10.6(2-39)	100
Topsmelt, *Atherinops affinis*	13	[1]13	[1]10(3-23)	[1]100
Goribaldi, *Hypsypops rubicunda*	20	19	31.2(1-144)	95
Halfmoon, *Medialuna californiensis*	13	12	19.8(1-75)	92
Opaleye, *Girella nigricans*	10	8	5.4(1-14)	80
Olive rockfish, *Sebastes serranoides*	11	5	2.6(1-4)	45
Blue rockfish, *Sebastes mystinus*	9	4	1	44
Sharpnose seaperch, *Phanerodon atripes*	13	5	2(1-5)	38
Senorita, *Oxyjulis californica*	36	9	11(1-59)	24
Sheephead, *Pimelometopon pulchrum*	14	2	1	14
Rubberlip perch, *Rhacochilus toxotes*	10	1	1	10
Cabezon, *Scorpaenichthys marmoratus*	10	1	1	10
Gopher rockfish, *Sebastes carnatus*	11	1	1	9
Pile perch, *Rhacochilus vacca*	15	1	1	7
Kelp rockfish, *Sebastes atrovirens*	16	1	1	6

[1] *Atherinops affinis* does not carry *C. hobsoni*, but rather is the sole host (with one exception, see text) of the very similar *C. serratus*.

Lepeophtheirus sp.; the other señorita carried a single *Lepeophtheirus* sp. Comparative data were obtained by examining 16 senoritas, 111 to 160 mm long, that had been cleaning. Of these, 11, or nearly 70%, carried copepod parasites on their external body surfaces: 6 carried from 1 to 59 *Caligus hobsoni*, 4 carried from 1 to 9 *C. serratus*, and 1 carried 3 *Lepeophtheirus* sp.

Significantly, those señoritas carrying *Caligus hobsoni* all had been cleaning blacksmiths, those carrying *C. serratus* had been cleaning topsmelt, and the one carrying *Lepeophtheirus* sp. had been cleaning a garibaldi. Thus the ectoparasites found on cleaning señoritas were in all instances forms that also infest the species which that particular señorita had been cleaning. The occurrence of *C. serratus* is especially interesting, because these señoritas are the only fish other than topsmelt found so far to carry this parasite.

Alerted to the phenomenon, I inspected the bodies of many señoritas that incidentally passed by during various phases of the work underwater. Ectoparasites were evident on some, but only on a small minority of the population. That the vast majority are not infested by such parasites accounts for the observation, noted above, that señoritas do not crowd around cleaners that initiate activity in their midst, as do blacksmiths, topsmelt, halfmoons, and others.

On the basis of these data, and on the general cleaning picture that has developed, I believe that at least most of the señoritas infested with caligid copepods are cleaners. Presumably they acquire these parasites while intimately associated with the former hosts during cleaning. That a given cleaner is found to carry parasites similar to those on the fish it has been attending, but no others, is further evidence that cleaning by individual señoritas tends to be species-specific.

Environmental Factors That Influence Cleaning

Temperature.—As noted above, the numbers of señoritas present at the 20- to 25-m station fluctuated in an apparent response to water temperature, with the critical level at about 12° to 13° C. Less cleaning occurred at lower temperatures (Figure 9), which would be expected with fewer señoritas present. Nevertheless, even considering the smaller numbers, the señoritas present at lower temperatures seem less active than those present at higher temperatures. The effect was striking on one occasion at 25 m when, with an influx of warm water, the temperature rose suddenly from 11° to 14.5° C. No change was noted in the numbers of señoritas present over this short period of time, but where no cleaning had been seen during a 20-min survey immediately before, shortly after the temperature rise six different groups of fishes being cleaned were in view simultaneously.

Turbidity.—When the water is turbid because of plankton or suspended sediment, there is noticeably less cleaning activity than when the water is clear. The fishes are generally more wary, and remain closer to cover when visibility is reduced.

Surge.—When there is a strong surge, a frequent occurrence, especially in water less than 10 m deep, there is far less cleaning activity than when the water is still.

Day-night.—The señorita, a strictly diurnal species that takes shelter under cover at night, does not clean after dark.

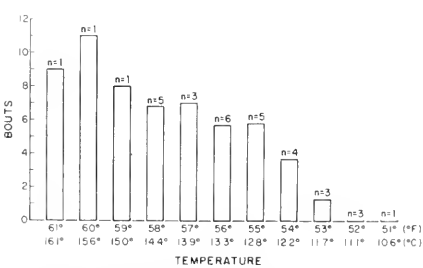

FIGURE 9.—Number of señorita cleaning bouts seen during each of 33 observation periods, 15-25 min long, at different water temperatures in an area 25 m deep at La Jolla. Periods during which temperature fluctuated were not considered. n = number of observation periods at that temperature; where $n > 1$, value given is the mean.

CLEANING ACTIVITY OF THE SHARPNOSE SEAPERCH

Unlike señoritas, which clean as adults as well as juveniles, all of the sharpnose seaperch that I observed cleaning were juveniles less than about 125 mm long. Occasionally noncleaning seaperch swim in groups of 15 or more, but those seen cleaning were always solitary, or in groups of two or three. In agreement with señoritas, cleaning seaperch do not establish well-defined cleaning stations, but instead may clean other fish at any point as they move from place to place. I found no evidence that fishes which are residents of other areas come to where seaperch are located for cleaning; rather, cleaning seaperch occur where resident fishes are numerous. As is true of señoritas, seaperch use the same picking technique to clean material from the bodies of other fish that they use to take small organisms from a benthic substrate. Clearly bottom-picking can be preadaptive to cleaning. Cleaning by seaperch, as by señoritas, usually occurs within 3 m of the substrate. However, there is little overlap in the cleaning areas of the two species: generally seaperch clean at greater depths and or in colder water than señoritas, where limited observations indicate they may predominate as cleaners even when señoritas are more abundant. Data illustrating this distribution of cleaning activity at a point in time were obtained at the 20- to 25-m and 30- to 35-m locations off La Jolla, where the two species co-occur (Table 1). Despite the fact that señoritas were observed to be far more numerous than perch throughout the depth range of this study (3-50 m), seaperch performed almost all the cleaning observed at the 30- to 35-m location, where cleaning by the much more abundant señorita was limited to a few isolated instances.

TABLE 4.—Number of bouts in which sharpnose seaperch and señoritas, respectively, were seen cleaning other fishes during 15-min observation periods at the 20- to 25-m and 30- to 35-m locations off La Jolla. Two observation periods, one at each location, and never more than 45 min apart, were made on each of the dates indicated.

Date	Number of cleaning bouts observed			
	20- to 25-m location		30- to 35-m location	
	Seaperch	Señorita	Seaperch	Señorita
22 Nov.	2	13	17	0
27 Nov.	0	7	1	0
9 Jan	2	5	9	0
15 Jan.	2	4	8	0
3 Feb.	2	12	9	2
Total	8	41	44	2

A measure of the incidence of cleaning individuals within the population of juvenile sharpnose seaperch was obtained during 39 observation periods at the 20- to 25-m and 30- to 35-m locations at La Jolla. These observations, totaling more than 26 hr, were made from September 1968 to February 1969. During this period, 201 juvenile seaperch were seen, of which 105, over 52%, were cleaning other fishes. Thus it appears that at least most sharpnose seaperch are cleaners when they are juveniles, whereas only a small minority of the señorita population seem to be cleaners.

Fishes Cleaned by the Sharpnose Seaperch

Because sharpnose seaperch were observed only at depths below 20 m, substantially less data are available on their cleaning activity than on that of señoritas. Of the 105 seaperch observed cleaning during the 39 observation periods reported above, all but one were cleaning blacksmiths; the lone exception was cleaning a solitary blue rockfish, *Sebastes mystinus*. On two other occasions, I saw sharpnose seaperch cleaning rubberlip perch, but otherwise the only fish seen being cleaned have been blacksmiths (Figure 10). Undoubtedly additional observations, especially in other areas, would expand this list. I observed señoritas cleaning in many different areas, but my observations of cleaning seaperch are limited to La Jolla. Clarke et al. (1967) saw a sharpnose seaperch cleaning a rockfish at 150 m off La Jolla, and Gotshall (1967) reported what he believed to be this species cleaning *Mola mola* off Monterey. Yet no matter how many different species the seaperch may in fact clean, there seems no doubt that blacksmiths are prime recipients in southern California, at least in depths shallower than 35 m.

FIGURE 10.—Sharpnose seaperch inspecting a blacksmith, which hovers to solicit cleaning.

Specific Cleaning Interactions—Seaperch-Blacksmith

The limited observations on cleaning by sharpnose seaperch provide details only on interactions with blacksmiths. As nearly as could be seen, when sharpnose seaperch clean blacksmiths the activity proceeds much as it does when blacksmiths are cleaned by señoritas, as described above. However, the observations were too few to determine whether or not cleaning activity is consistently initiated by the cleaner. Several times blacksmiths hovered in their typical head-down posture before seemingly unresponsive seaperch, but perhaps the seaperch had earlier made some initial gesture. Whenever it could be determined, the seaperch initiated the cleaning.

Some details were obtained at the 20- to 25-m location at La Jolla, where two seaperch, known to have been cleaning blacksmiths, were each kept under surveillance for 15 min, while their activity was monitored on tape. Both swam on irregular courses among the rocks but remained within an area encompassing about 15 to 20 m^2. During this time one entered into 4, the other 5, separate cleaning bouts, averaging 2.6 (range 0.5-7.5) and 1.8 (range 0.75-3.5) min long, respectively, all with blacksmiths. The cleaner initiated the activity in each instance, but immediately thereafter a number of other blacksmiths converged on the spot. Most of the cleaning bouts continued after the original blacksmith had left the group, and a succession of others arrived and departed before the bout ended. Although usually they hovered head-down before the cleaners, the blacksmiths nevertheless assumed a wide variety of attitudes. During much of the time they swam with the blacksmiths, the two seaperch under surveillance closely inspected the blacksmith's bodies and actually picked at them 18 and 14 times, respectively. Most of the cleaning was directed at the fin bases, particularly the caudal. While in company with the blacksmiths, one of the seaperch broke away from the group and swam to look closely at the dorsal fin of a blue rockfish. However, no cleaning occurred: the blue rockfish swam away as though uninterested in cleaning and the seaperch returned to the blacksmiths. When not in company with the blacksmiths, the two seaperch swam alone 1 or 2 m over the substrate. One descended to the bottom twice and picked at gorgonians: five times on the first descent, once on the second.

Once a blacksmith was seen obviously attempting to present its caudal fin to a seaperch, without success in enticing the seaperch to clean. Close inspection did not reveal parasites, but part of the fin was torn away and shredded flesh was exposed. Apparently this blacksmith was presenting a point of irritation to the cleaner, which in this instance was an injury, not a parasite. Some cleaners, for example, *Abudefduf troschelii*, which picks molting skin from the Galápagos marine iguana (Hobson, 1969b), will clean dead or injured itssue, but at least on this occasion the seaperch showed no interest.

Material Removed from Other Fish by the Sharpnose Seaperch

To determine the food of cleaning seaperch, I examined the gut contents of 16 specimens, 74 to 122 mm long, that were speared as they cleaned blacksmiths. Food items in their guts, ranked as percentage of each item in the entire sample, were as follows: caligid copepods, 68%; caprellid amphipods, 16%; gnathiid isopod larvae, 9%; algae, 1%; and unidentified items, 6%. Thus ectoparasitic caligids and gnathiids made up 77% of the material. All 16 specimens contained ectoparasites; in fact, ectoparasites constituted the vast bulk of the material in all but one individual, which had fed more heavily on caprellids. As with señoritas, when an appreciable amount of free-living material was present, it was usually sharply divided from the ectoparasites and more digested to the rear in the digestive tract. All the identifiable caligid copepods among this material were *Caligus hobsoni*, which is consistent with what is known of ectoparasites on blacksmiths, the species cleaned by these seaperch, and indicates feeding habits similar to those of the cleaning señorita, presented above.

Incidental Cleaning by a Close Relative

Although sharpnose seaperch were not seen in water less than 20 m deep, the white seaperch, a very similar species, is frequently abundant there. The white seaperch was probably the most numerous of the embiotocids during most of the observations made at the 3- to 10-m location off La Jolla. Underwater the white seaperch and the sharpnose seaperch are nearly identical, but can be distinguished by the dusky bordered caudal fin of the former and the black-tipped pelvics and more pointed snout of the latter.

White seaperch are especially abundant in groups of 10 or more close to surfgrass in 3 or 4 m of water off La Jolla. Typically they hover head-down; in this attitude they are not soliciting cleaning but rather are intently regarding the surface of the vegetation, at which they pick occasionally. Tiny organisms that live on the surfgrass are prey of these fish: five white seaperch, 80 or 81 mm long, speared in this habitat, were filled with (showing percent of total volume) caprellid amphipods (80%), gammarid amphipods (5%), isopods (2%), fragments of algae with encrusting bryozoans (10%), and unidentified crustacean parts (3%). Quast (1968) found that specimens from a kelp bed had fed mostly on small bottom-dwelling crustaceans, polychaetes and bivalves, as well as kelp fragments, some of which were heavily encrusted with bryozoans. Thus the bottom-picking feeding habits of the white seaperch are very similar to the noncleaning habits of the sharpnose seaperch.

On one occasion, I saw a white seaperch swim 1 or 2 m above the surfgrass in company with a lone blacksmith, which hovered head-down in the manner typical of one that desired to be cleaned. The white perch picked at the blacksmith's body several times, but the bout was brief, and the perch soon joined a group of 8 to 10 others of its own kind near the surfgrass below. This seaperch, which proved to be 79 mm long, was speared, and its gut contents included 58 caprellid amphipods, a single gammarid amphipod, one small isopod, plant fragments with encrusted bryozoans, and some unidentified nonparasitic crustacean remains. No ectoparasites were found; its food was similar to that of the other white seaperch reported above. On another occasion I saw a white seaperch cleaning several blacksmiths over a sandy bottom in 12 m of water, but this individual was not collected. Probably the observed cleaning was no more than a brief incidental activity for these fish. At no other time did I see any indication of cleaning by this species, but perhaps the activity is more frequent under appropriate conditions.

CLEANING ACTIVITY OF THE KELP PERCH

Because the kelp perch is not abundant in the La Jolla study area, where larger kelps are sparse, most observations of cleaning by this fish were made incidentally during other projects in areas heavily forested with kelp. However, these other projects generally were centered on the sea floor, whereas kelp perch concentrate

above in the midwater and canopy regions. Nevertheless, observations of cleaning were sufficiently frequent to recognize this species as a habitual cleaner, though probably less so than either the señoritas or the juvenile sharpnose seaperch. In taking material from the bodies of other fishes, the kelp perch uses the same picking technique that it employs to pick items from an algal substrate, or that are adrift in midwater. Its pointed snout and dentition, which is similar to that of the señorita, as described above, are well suited to cleaning.

Insofar as an aggregation of kelp perch tends to remain in one location, these fish can perhaps be regarded as maintaining a station at which other fishes are cleaned. But I saw no indication that more than one or a few members of a given aggregation clean, or that other fishes come to these locations from any distance for cleaning. In fact, I saw only blacksmiths and other kelp perch being cleaned by this fish. In the one observation of intraspecific cleaning, a single kelp perch swam among others of its aggregation, intently inspecting their bodies. Usually the subject of this attention moved away, whereupon the cleaner moved to another fish. A few responded to the cleaner by erecting their fins and hovering immobile in a head-down posture, and these were cleaned. Occasionally a fish being cleaned suddenly darted away as if the cleaner had been too vigorous in its attentions. All blacksmiths being cleaned were solitary individuals that hovered in head-down soliciting fashion close to an aggregation of kelp perch. Whether or not one of the perch had earlier made an initiating overture was never determined. Never more than one or two of the perch in the aggregations were seen cleaning these blacksmiths. Occasionally a cleaner would closely follow a halfmoon or kelp bass that incidentally passed close by, but I saw no evidence that these fish were interested in the perch, and no cleaning occurred.

Three kelp perch, 91 to 99 mm long, one of which had been cleaning a blacksmith, were collected from an aggregation hovering near a stand of feather-boa kelp. The gut contents of the individual known to have cleaned the blacksmiths contained (showing the percent of total volume): gnathiid isopod larvae (50%), nonparasitic isopods (5%), gammarid amphipods (5%), caprellid amphipods (20%), and unidentified material (20%). Neither of the two that were not known to have cleaned contained evidence of ectoparasites: one was full of caprellid amphipods (90%) and unidentified material (10%), whereas the other had nothing in its digestive tract except a few unidentified fragments posteriorly.

Limbaugh (1955) reported kelp perch cleaning kelp bass, opaleyes, garibaldis, blacksmiths, and walleye surfperch (*Hyperprosopon argenteum*).

DISCUSSION

Various cleaning fishes remove a wide variety of deleterious material from the bodies of the animals they service. In addition to ectoparasites, this material includes diseased, injured, or necrotic tissue, fungi, and unwanted food particles (Feder, 1966; Hobson, 1968, 1969b; and others). However, the discussion below considers cleaning only as the removal of ectoparasites, because my data indicate that these are the only items taken in significant amounts from California fishes by the cleaners considered in this report.

INCIDENTAL VS. HABITUAL CLEANING

Cleaning is widespread among small-mouthed marine fishes that characteristically pick minute organisms from the substrate (Hobson, 1968). Included are species of the families Chaetodontidae, Pomacentridae, Labridae, Embiotocidae, Blenniidae, and others. Morphological and behavioral characteristics suited to their way of life have preadapted many species of these families for the cleaning habit. Probably some such fishes pick ectoparasites only incidentally during routine foraging when under certain conditions the body of an adjacent fish, infested with ectoparasites, becomes accessible as just another feeding substrate. The relative tendency of a given species to clean likely is influenced by both short-term and long-term environmental changes. Such changes may be expected to alter

interspecific relations, by affecting not only the relative availability of various prey organisms and the incidence of various ectoparasites, but also the species composition of the interacting fishes themselves.

In California the white seaperch likely is one of those species that cleans only occasionally as an incidental adjunct to regular foraging. Several other California species reported by Limbaugh (1955) and Gotshall (1967) clean, including black perch, pile perch, and rainbow seaperch, but they have not been seen doing so by me. The report of cleaning by the blacksmith (Turner et al. 1969) remains an anamoly, as this fish does not fit the pattern of a bottom-picking predator described above. However, it may be significant that many of those substrate-picking predators which clean most frequently are species that also feed on material adrift in midwater, as do the señorita, sharpnose seaperch, and kelp perch. Thus this mode of feeding too, including the taking of plankton, may, in some species, favor adaptations that are suited to cleaning. Fishes that are adapted to both substrate-picking and plankton-picking may possess adaptations especially well suited to cleaning.

Probably many species of fishes clean incidentally on isolated occasions, but relatively few are habitual cleaners. And even the habitual cleaners vary greatly in the degree to which they are specialized for this habit. Species of the Indo-Pacific labrid genus *Labroides* are highly specialized cleaners that feed almost exclusively on ectoparasitic crustaceans (Randall, 1958; Youngbluth, 1968). These fishes possess many specific morphological and behavioral specializations that are adapted to this way of life (Feder, 1966; Losey, 1971). However, only a small minority of cleaners are so highly specialized; most are but part-time practitioners of the cleaning habit, with much of their food being derived from other sources.

That some cleaners depend on ectoparasites for prey, whereas others can subsist equally well on food from other sources, has led to classifying various species as either obligate or facultative cleaners (e.g., Youngbluth, 1968). The señorita, sharpnose seaperch, and kelp perch may well resist being so classified because their cleaning seems to be characteristic not so much of a species as of just certain individuals. At least at a given time, most señoritas do not clean, whereas some seem to be facultative cleaners, and a few might even be obligate cleaners. Juvenile sharpnose seaperch follow a similar pattern, but with a relatively higher incidence of individuals that clean. Limited data can only suggest that the status of the kelp perch may be similar.

CLEANING INITIATED BY THE SEÑORITA

Usually there seem to be fishes present that need cleaning, as shown when a señorita identifies itself as a cleaner by initiating action with, say, a blacksmith or a topsmelt, and immediately is converged upon by many other fish that crowd in its way seeking attention. That such fishes generally wait for a señorita to begin the cleaning, rather than attempting to initiate activity themselves with one of the many señoritas present, likely reflects a low probability of success if they make the first move. If, as it seems, the vast majority of señoritas are not cleaners, or at least not currently predisposed to clean, then random efforts to solicit service would not seem adaptive.

This situation contrasts with that of the Hawaiian wrasse *Labroides phthirophagus*, of which all individuals seem to be obligate cleaners (Youngbluth, 1968), and which is not nearly as abundant on Hawaiian reefs as the señorita is in California. In centering their activity around well-defined stations, the distinctive *L. phthirophagus* can be recognized readily by others that need cleaning. Thus, not surprisingly, cleaning encounters that involve *L. phthirophagus* are regularly initiated by fishes seeking cleaning (Losey, 1971).

We have seen that under certain circumstances various fishes initiate cleaning encounters with señoritas. Some fishes successfully do so by hovering amid unusually dense concentrations of señoritas, but the overtures of such fish are not directed at individuals; rather, they are broadcast to the assemblage at large. The success of this tactic presumably follows the probability that an individual predisposed to clean occurs among such a large number of señoritas.

Kelpfish regularly solicit cleaning from individual señoritas, but the situation is exceptional. Because kelpfish rise into midwater for cleaning, it appears that they do not receive satisfactory service in their regular habitat amid benthic vegetation. In their usual surroundings, where they are extremely difficult to discern, the cryptic kelpfish may be relatively inaccessible to cleaning señoritas. One can see why a fish thus handicapped might be required to initiate needed cleaning itself. The number of unsuccessful attempts experienced by kelpfish before a señorita was finally induced to clean them underscores the existing problem of locating a cleaning individual.

SPECIES-SPECIFIC CLEANING

Because the cleaning señorita initiates most of its activity, it has the opportunity to select its clients, and the data indicate that a species-specific choice is exercised. That individual cleaners tend to limit their selection to members of only one species may be related to the fact that they initiate cleaning on the home ground of the fishes they serve, when these fishes are engaged in some of their regular activity. As each of these clients has distinctive habits, a señorita approaching to clean a fish of one species faces a somewhat different situation than a señorita approaching to clean a fish of another species. The distinctions often are subtle, but may be significant enough to account for a given señorita's tendency to seek out members of only one species.

Again we can draw a contrast with the cleaning behavior of *Labroides phthirophagus*, individuals of which receive members of many different species at well-defined cleaning stations (Randall, 1958; Youngbluth, 1968). Probably such nonspecific cleaning is characteristic of cleaners whose activity is confined to these established locations. Fishes that visit such cleaning stations enter the cleaner's own territory, and frequently join a mixed-species group that hovers in wait for service. In tending these fishes on its home ground, the cleaner is receiving them on its own terms, so to speak, so that the situations surrounding cleaning bouts with all of the different species are essentially the same.

Cleaning by the señorita may not be species-specific on those few occasions when the cleaning activity is initiated by the fish in need of such service, for example by the kelpfish, as described above. Although they show some difficulty locating a receptive señorita, kelpfish nevertheless seem far more successful at doing so than one would expect if indeed they are required to find one that will clean only kelpfish. Thus, although individual señoritas seem to be species-specific when they themselves initiate cleaning, they may be considerably less so, and perhaps even nonspecific, when the other fish makes the initial overture. There are no data on this point, however.

The extent to which these considerations apply to juvenile sharpnose seaperch and kelp perch cannot be ascertained because data are lacking.

SIGNIFICANCE OF POSTURES ASSUMED BY FISHES THAT SOLICIT CLEANING

When members of an assemblage of fishes like blacksmiths or topsmelt converge on a cleaning site that has developed in their midst, probably their attention was initially alerted by the unnatural-appearing posture assumed by the individual first approached by the cleaner. Usually this posture does not seem to be assumed purposefully, but rather results when the fish, having ceased swimming and immobilizing its fins, passively drifts out of its regular attitude (Hobson, 1965b). The posture thus assumed varies, especially between species, where perhaps differing centers of gravity are determining factors. Thus the blacksmith usually hovers head-down, whereas the topsmelt is more often tail-down. Sometimes an unnatural-appearing posture is actively assumed when the fish attempts to present to the cleaner a certain part of its body, presumably that part carrying an irritation. By virtue of their unusual appearance, these postures in cleaning interactions serve to draw attention to the fish that is cleaned. It does not seem necessary that any particular posture be assumed, only that it look out of the

ordinary. Reports are widespread (see Feder, 1966) of cleaning recipients assuming these unnatural-appearing postures.

Attention-getting postures assumed by fishes being cleaned probably occurred incidentally during the early development of cleaning symbiosis, when fishes hovering to be cleaned quite naturally stopped moving and passively drifted out of their regular attitudes. As the various cleaning relations evolved, apparently this obvious cue subsequently assumed a different role as a signal in different situations. Generally these postures are suggested to be signals between the recipient of cleaning and the cleaner, indicating a readiness to be cleaned. Quite likely this is the primary signal-function in activity involving such cleaners as *Labroides phthirophagus*, where all members of the species are cleaners and where activity is centered around cleaning stations that are well known to other fishes in the area. In this situation a fish in need of cleaning should be reasonably successful in advertising its condition by assuming the characteristic soliciting posture before a fish recognizable as a cleaner. Losey (1971) showed that various fishes regularly employ this tactic to induce *L. phthirophagus* to clean them. Observations in the Gulf of California demonstrated that the cleaning station itself has played a role in establishing the soliciting posture as a cue. There I have seen the goatfish *Mulloidichthys dentatus* hovering head-down at cleaning stations of the butterflyfish *Heniochus nigrirostris*, when the resident cleaner was itself temporarily absent. Losey (1971) observed similar behavior among Hawaiian fishes. In such a situation the hovering posture probably alerts the cleaner to fishes that have arrived for cleaning.

However, in cleaning activity involving the señorita, the soliciting posture usually is assumed only after cleaning has been initiated by the cleaner. The problem of recognizing an individual that will clean among the vast majority of señoritas that do not clean, coupled with the absence of well-defined cleaning stations, would reduce the adaptiveness of the client's soliciting posture as a cue to initiate cleaning. Probably the most effective way for a fish to obtain needed cleaning in this situation is to wait until a cleaner has identified itself by initiating activity with some fish in the area. Once this has occurred, one can see the value of the posture, when assumed by the first fish to be approached, as a cue in alerting other fish that need cleaning to the presence of a cleaner. In effect, then, the fish assuming the soliciting posture advertises the temporary existence of the transient cleaning "station" to other potential recipients of cleaning. Well-defined cleaning stations like those of *Labroides phthirophagus* do not need this sort of advertisement, as their locations are well known to the fishes that visit them. Nor is it necessary that cleaning individuals of *L. phthirophagus* be pointed out, as all members of that distinctive species are cleaners. Despite this, it is probable that fishes hovering to be cleaned at a *Labroides* station themselves create a visual cue that tends to attract other fishes.

There may also be a maladaptive aspect to the postures assumed by fishes that solicit cleaning. In hovering at an unnatural angle, fins immobile and erect, a fish may enhance its chances of being cleaned, but it would also seem likely to draw the attention of predators and to handicap itself in evading attack. Perhaps such an increased vulnerability accounts at least in part for the sharp decline in cleaning that occurs with reduced visibility, when predators can approach closer undetected. Increased vulnerability may also account for the observation reported earlier (Hobson, 1965c), where pomadasyids in the Gulf of California abruptly broke away from cleaners when a predator approached.

THE POSSIBILITY THAT FISHES BEING CLEANED EXPERIENCE STRESS

Being prodded and picked over by an animal of another species would seem to require a difficult adjustment for a fish. It may well be that fishes experience stress under this circumstance, even when the behavior is well established. Certainly observations have shown that this experience can be uncomfortable, judging from how often fishes being cleaned suddenly bolt forward, and swim away, apparently having been nipped too vigorously by the attending cleaner. Sometimes too, a fish approached by a cleaner clearly

experiences conflicting responses, one moment tolerating or even soliciting the cleaner's attentions, and the next moment chasing it away on each approach. Such ambivalent behavior was especially evident in rubberlip perch. Losey (1971) noted that *Labroides phthirophagus* in Hawaii is sometimes attacked by fishes that it attempts to clean, and suggested that this may occur when the cleaning is painful to the host fish.

The color changes shown by many fishes being cleaned (Randall, 1958; and others) may in fact be manifestations of stress. It is well known that many fishes experience color changes in response to stress. Earlier (Hobson, 1965a) I discussed the striking color change of the goatfish *Mulloidichthys dentatus* when it solicits cleaning in the Gulf of California, and pointed out that this fish shows the same color change in other situations that are obviously stressful. Such color changes have been regarded as signals between the fishes being cleaned and the cleaners, (e.g., Feder, 1966), functioning in the cleaning interaction much like the soliciting attitudes discussed above. As with the attitudes, any role such color changes may now have assumed as a signal probably evolved from an incidental by-product of early cleaning. I have no data on this point relating to the California species, as such color changes are not especially evident in fishes that were observed being cleaned there.

ARE CLEANERS IMMUNE FROM PREDATION?

Reportedly some cleaners are immune from predation because of the service they provide the predators (Feder, 1966; and others). Limbaugh's (1961) belief that the señorita enjoys such immunity is based on observations of this labrid entering the open mouth of kelp bass to clean and on not finding it among the stomach contents of predators during a food-habit study. However, Quast (1968) found señoritas in the stomachs of kelp bass, and H. Geoffrey Moser, U.S. National Marine Fisheries Service (unpublished data), found señoritas in stomachs of the bocaccio, *Sebastes paucispinis*, and the starry rockfish, *S. constellatus*.

I doubt that cleaners enjoy immunity in the sense that predators, recognizing them as benefactors, actively avoid preying on them. Cleaners may recognize those predators which are not at that time intent on feeding and may restrict their cleaning to such individuals. A predator that assumes a soliciting posture may effectively advertise this situation, and no doubt other cues exist. Such mechanisms would reduce the chance of cleaners placing themselves in vulnerable situations while cleaning. In addition, cleaners probably are not as vulnerable while cleaning large predators as might be expected simply because cues characteristic of feeding situations are not present. In associating themselves so intimately with predators, cleaning fishes show behavior that is so unlike that of prey that predators probably do not regard them as food. However, even if such factors do reduce the danger that might seem inherent in the cleaning act, I doubt that their cleaning role affords these fishes any security from being eaten in noncleaning situations.

PARASITES AS PREY OF THE CALIFORNIA CLEANERS

It is hardly surprising that the fishes which are cleaned most frequently in California are those which are the most abundant and at the same time carry the most ectoparasites. Thus the blacksmith, topsmelt, halfmoon, and garibaldi are the fishes cleaned most frequently, and the survey of ectoparasites showed them to be among the most heavily parasitized. The vast majority of ectoparasites on these particular fishes are mobile forms, mostly caligid copepods and gnathiid isopod larvae, that occur on the body surface of their hosts. That these same parasites were found to make up the diet of the cleaners attending these fishes is consistent with the observation that only the exteriors of fishes were seen being cleaned during this study.

Although the forms infesting the external body surface are the most numerous ectoparasites on the fishes available to the California cleaners, many other types were found to infest the oral and branchial cavities. One might question why these other parasites do not seem to be

taken, especially as Limbaugh (1955, 1961) reported señoritas entering the mouth of the kelp bass and cleaning beneath the gill covers of the garibaldi. Furthermore, such behavior has been widely reported for some other cleaners, such as species of *Labroides* (Eibl-Eibesfeldt, 1955; Randall, 1958; and others), and some echeneids are known to habitually feed on copepods from the branchial cavities of sharks (Cressey and Lachner, 1970). Nevertheless, any such activity by señoritas must be relatively rare. In discussing this situation I limit my remarks to the señorita, because data are presently insufficient to determine whether the same may apply to the sharpnose seaperch and kelp perch.

Señoritas would not be expected to take parasites from the oral or branchial cavities as often as species of *Labroides* or echeneids if for no other reason than they simply are too large relative to most of the fishes they clean. Whereas species of *Labroides* or the echeneids are small enough to enter the oral and branchial cavities of most of the fishes they service, the señorita is nearly as large, and sometimes even larger, than most of its clients. Significantly, Limbaugh observed señoritas cleaning within the oral and branchial cavities of kelp bass and garibaldis, both of which are relatively large species. Most of the señorita's cleaning is directed toward smaller species, like the blacksmith and the topsmelt.

The specialized techniques that would be required to prey on the parasites of the oral and branchial cavities would probably pose another problem to the señorita. In its regular habit of taking parasites from the external surfaces of fishes, the cleaning señorita concentrates on just a few forms that not only are numerous on many of the most abundant fishes, but also are not too dissimilar from free-living prey of the species. Sometimes these external forms also occur in the branchial cavity, and some similar forms, e.g., homolochids (Figure 7), habitually occur there and in the oral cavity. But the majority of parasites characteristic of the branchial and oral cavities are aberrant forms, e.g., dichelesthiids, chondracanthids, and lerneopodids (Figure 7), and these are unlike anything else encountered by the señorita. No one type predominates; rather, they occur in a wide variety of forms, none widespread among the different species of fishes, and none especially abundant (except on an occasional individual fish). Thus a cleaner probably could not subsist on one type alone but would have to master a repertoire of specialized techniques in order to exploit enough of these varied forms to make it worthwhile. And before access is gained to the site of infestation, a much more refined cleaner-host interaction must have evolved than is necessary when parasites are simply cleaned from the external body surface. No such relation would evolve unless the cleaner acquired the precise manipulations necessary to pick attached parasites off the gills without damaging the delicate gill membranes. Obviously the cleaning relation would not be adaptive if such damage occurred. In short, to feed habitually on parasites from the oral and branchial cavities would seem to require a higher degree of specialization than has been demonstrated by the señorita. It seems unlikely that such specialization would develop as long as the more abundant and readily available forms on the body surfaces continue to satisfy the cleaning needs of the species. Certainly judging from the way blacksmiths, topsmelt, and other fishes vigorously compete to have their external parasites removed, it would seem that there is little immediate chance of these parasites falling into short supply.

CLEANING SYMBIOSIS AND THE DISTRIBUTION OF SHORE FISHES

In his often-cited report on cleaning symbiosis, Limbaugh (1961: 48) stated:

> In my opinion it is the presence of the señorita and kelp perch that brings the deep-water coastal and pelagic fishes inshore to the edge of the kelp beds on the California coast. Most concentrations of reef fishes may similarly be understood to be cleaning stations. Cleaning stations would therefore account for the existence of such well-known California sportfishing grounds as the rocky points of Santa Catalina Island, the area around the sunken ship *Valiant* off the shore of Catalina, the La Jolla kelp beds and submarine canyon and the Coronado Islands.

Presumably this conclusion was intuitive, as no

data were presented. In his review of cleaning symbiosis, Feder (1966: 368), basing his conclusion on Limbaugh's work, similarly stated: "In all probability, many good fishing grounds are such primarily because they are cleaning stations." I believe that this contention is unfounded. Señoritas are the major cleaners in California inshore waters, so that if cleaning symbiosis does account for most concentrations of reef fishes in this region, as Limbaugh suggested, then señoritas would be the cleaner largely responsible. Cleaning occurs wherever señoritas are concentrated but clearly is not a major activity of the population, even though it may be so for a relatively few individuals. In any event, it seems safe to conclude that cleaning is not among the major factors determining the distribution of señoritas. And if cleaning does not determine the distribution of señoritas themselves, it seems unlikely that it would determine the distribution of other species.

Undoubtedly many factors contribute to creating situations that draw concentrations of fishes to certain locations. Where a number of different species have similar requirements, assemblages will develop where conditions satisfying these requirements are optimum. The presence of these fishes increases the complexity of the environment, thus creating situations that support still other species, and so on. Often it is apparent that certain features are especially significant as a basis for these concentrations. Consider, for example, the rocky points that Limbaugh included in his list of "well-known California sport-fishing grounds." The flora and fauna of these locations are generally rich, a fact probably related to such local features as converging currents that frequently produce upwelling and nutrient-rich waters. Plankton is commonly abundant here, along with plankton-feeding fishes like the blacksmith. Señoritas and other species frequently are numerous here too, but the main attraction seems to be a generally rich food supply rather than available cleaning. Similarly it is unrealistic to attribute concentrations of fishes around sunken ships to cleaning activity. Where a wreck has settled on an open expanse it becomes a haven for fishes that require a nearby structure for cover or a spatial reference point. Obviously such fishes will center themselves here, because the surrounding featureless substrate does not meet their requirements. I am describing a well-known phenomenon, one that is the rationale behind constructing artificial fishing reefs. Thus food and a suitable substrate often appear to be key features in a habitat that supports large numbers of fishes. Of course to cite just one or the other would be an oversimplification, as requirements in both must be satisfied, along with many other perhaps more subtle needs. The various species assembled at such locations interact in a variety of ways; cleaning symbiosis is one such interaction, and undoubtedly an important one, but hardly the prime reason for them being there.

CHANGES IN HABITS WITH TIME

Uncertainty remains regarding changes in habits with time. The picture of activity developed in this report was derived directly by observing activity and also indirectly by examining both digestive-tract contents and the specific ectoparasites that infest the various fishes. But these methods only define situations that exist over a relatively brief span of time. Data on individual activity over longer periods are needed. Certainly habits of individuals change with time, but how much change and over how much time? The fact that material in the digestive tracts frequently occurs in sharply delimited homologous blocks indicates that these fishes often feed heavily or even exclusively on one particular type of prey, and then abruptly shift to something else. Are habits such as a relative tendency to clean and to clean members of just one species immutable characteristics of individuals, or have the observations described in this report simply defined temporary situations that the various individuals just happened to be experiencing at the time they were singled out for study? It is possible that all señoritas clean at one time or another, though not all at once, and only a few at a time. It is also possible that a señorita, which tends to clean members of just one species during a given period of cleaning, may select members of another species during

a subsequent period of cleaning. Despite these questions, the conclusions drawn in this report are dependent only on an accurate assessment of the immediate situation, so that their validity is not affected by whether or not the habits of individuals under study remain basically unchanged over time.

A NOTE ON INDIVIDUAL VS. SPECIES HABITS

Information on variations in feeding behavior among individual fish under natural conditions is difficult to acquire. Typically a given behavior is described as a species characteristic, and the extent to which this behavior varies among the different members of the species is unknown. Observations of cleaning by the señorita demonstrate that different individuals in a population may react differently to a given situation. Unquestionably this phenomenon extends beyond cleaning behavior to other facets of the animal's activity. If, as is probable, some of the characteristics of individual fish result from early imprinting, then different members of the same population could be expected to react differently in certain situations throughout life. In any event, it seems unquestionable that the behavior of an individual is considerably more limited than that descriptive of its species, or even its own population.

CONCLUSIONS

1. Three inshore species of fishes in southern California are habitual cleaners: the señorita, the sharpnose seaperch, and the kelp perch. A number of other species clean occasionally as an incidental adjunct to their regular feeding.

2. The señorita may clean throughout its postlarval life, whereas cleaning by the sharpnose seaperch is an activity largely of juveniles. The life-history period during which kelp perch clean has not been defined.

3. Cleaning is of secondary significance to these species, although it may be of major significance to certain individuals. Only a few of the many señoritas present at a given time clean, and the same seems to be true of the kelp perch. The incidence of cleaners is much higher among juvenile sharpnose seaperch, but the adults of this species do not seem to clean regularly. The major food of all three species is free-living organisms which they pick from a substrate and midwater.

4. There is little overlap between the cleaning areas of the three species. The señorita is the major cleaner in southern California inshore waters by virtue of its great abundance in a variety of rocky habitats. However, the kelp perch may be the predominant cleaner in the canopy region of the kelp beds, where the species concentrates, and the sharpnose seaperch is the predominant cleaner where it occurs at depths below about 20 to 30 m and/or water under 12° or 13° C, even though the señorita may be more abundant.

5. The señorita and sharpnose seaperch do not establish well-defined stations at which they receive other fishes seeking to be cleaned—a situation frequently described for other cleaner fishes. Rather, as they move from place to place, individuals of these species approach and clean other fishes in various different locations.

6. Cleaning activity by these species is essentially limited to removing ectoparasites from the external body surfaces of fishes. They do not ordinarily take parasites of the oral and branchial cavities. The dentition of the señorita and kelp perch, which is similar and which includes a number of long, curved canines that project forward at the front of each jaw, seems especially suited to pick ectoparasites.

7. The major prey taken by these fishes through cleaning are caligid copepods and gnathiid isopod larvae. The species of parasite taken most often by the señorita and sharpnose seaperch is *Caligus hobsoni*.

8. Some species of fishes are cleaned far more often than others, and many species that cooccur with these cleaners are not cleaned at all. The fishes most frequently cleaned are those which at the same time are most abundant and most heavily infested with ectoparasites. The most numerous ectoparasites on these fishes are caligid copepods, the most abundant of which is *C. hobsoni*.

9. Cleaning effectively reduces the number of ectoparasites that infest the external body surfaces of fishes that interact with the cleaners.

10. At any given time, many individuals of the more frequently cleaned species are in need of cleaning. Nevertheless, in activity involving the señorita, infested fishes do not ordinarily attempt to initiate cleaning but instead wait for cleaning to be initiated by the cleaner. Because the vast majority of señoritas are not cleaners, or at least are not currently predisposed to clean, random efforts to solicit cleaning from señoritas in the population at large would not be adaptive.

11. When initiating its cleaning activity, a given individual señorita tends to approach and clean members of only a single species of fish.

12. Because the vast majority of señoritas are not currently predisposed to clean and because there are no well-defined cleaning stations, the unnatural-appearing posture assumed by a fish approached by a cleaner is an important cue in advertising the location of available cleaning to other fish in need of this service.

13. Fishes being cleaned probably experience some degree of stress. The color changes exhibited by some fishes when being cleaned are essentially manifestations of this stress; secondarily, they may have assumed a signal-function in certain cleaning interactions.

14. While intimately associated with the fishes they clean, señoritas frequently become infested themselves by the same parasites they are attempting to remove from these other fishes.

15. Cleaning activity is sharply curtailed when visibility is reduced by turbid water or when there is strong water movement, such as a heavy surge.

16. Cleaning activity among these fishes is a diurnal phenomenon. There is no evidence that it continues after dark.

17. Any so-called "immunity" from predation that a cleaner may enjoy probably relates (1) to an ability to recognize predators that are not intent on feeding and to limit cleaning to such individuals, and (2) to the fact that behavior exhibited by a cleaner servicing a predator is so unlike that of prey that the predator does not regard the cleaner as food. However, their role as cleaners probably does not afford these fish any security from being eaten during noncleaning situations.

18. Cleaners are widespread among small-mouthed marine fishes that characteristically pick tiny organisms from a substrate. This mode of feeding, especially when combined with the capacity to pick tiny prey that are adrift in midwater, preadapts fishes to the cleaning habit.

19. There is no basis for the contention that many of the good fishing grounds in southern California are such because fishes have congregated to be cleaned by resident cleaners.

20. Feeding behavior varies significantly among individuals of at least some species. Thus the habits of an individual can be more limited than those descriptive of its species or even its own population.

ACKNOWLEDGMENTS

Lloyd D. Richards assisted me in collecting the data for this study. I thank Roger F. Cressey and Thomas Bowman, U.S. National Museum, who identified the parasitic copepods and isopods, respectively, around which much of this study is centered. I thank, too, Carl L. Hubbs and Richard H. Rosenblatt, Scripps Institution of Oceanography, and John R. Hunter, U.S. National Marine Fisheries Service, for helpful comments on a draft of the manuscript.

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GRAY WHALES, *Eschrichtius robustus*, AVOID THE UNDERWATER SOUNDS OF KILLER WHALES, *Orcinus orca*

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ABSTRACT

Underwater sound playback experiments were undertaken to determine if gray whales would avoid the sounds of killer whales. When presented killer whale "screams" from an underwater projector, the gray whales swam directly away from the sound source. Controls of no intended stimulus, pure tones, and random noise generally failed to induce an avoidance. It appeared that gray whales localized the killer whale sounds and avoided them as a sign of potential danger.

Killer whales, *Orcinus orca*, are known to attack large marine mammals, including gray whales, *Eschrichtius robustus* (Scammon, 1874; Morejohn, 1968). Several accounts of such attacks were related to the first author by observers who witnessed these events. Among these were Dr. Carl L. Hubbs of Scripps Institution of Oceanography, University of California, and Alan Baldridge, Hopkins Marine Station, Stanford University. In January 1952, Dr. Hubbs saw a gray whale that swam into the thick kelp beds off La Jolla, Calif., apparently fleeing from a group of killer whales. Off the Point Lobos State Reserve, Carmel, Calif., Baldridge observed a gray whale calf being eaten by six to seven killer whales. Other observers had seen the initial attack which also involved the mother whale. When Baldridge arrived, the killer whales were chewing on the lips, tongue, and throat of the dead young gray whale.

Killer whales produce a variety of underwater phonations, including high pitched "screams" and trains of well-separated clicks (Schevill and Watkins, 1966). Underwater sounds have been recorded from about one-half of the known species of marine mammals, and probably all are capable of some vocal behavior. However, except for the echolocating abilities of porpoises and a few of their own behaviors associated with sound production, very little is known about the significance of underwater sound to marine mammals—virtually nothing where the large whales are concerned.

Taking advantage of the gray whales' migration in nearby waters, we conducted underwater sound playback experiments to find out if they would react to the underwater sounds of killer whales, possibly avoiding them as a sign of danger.

METHODS

The experiments took place off Point Loma, San Diego, Calif., during successive migrations of gray whales in January 1969 and 1970. Each year about 11,000 of these whales pass San Diego on their southward migration to the breeding grounds off Baja California and the Mexican mainland (Rice, 1970). The whales, some with calves, return to our waters in the early spring on their way back to the Bering Sea and the Arctic Ocean.

Field work was done from a large catamaran, RV *Sea See*, which served as a stable and roomy platform, moored in 30 m of water, 33 m seaward of an extensive kelp bed. The ship held a northerly heading at the mooring. Gray whales normally funneled through this location staying relatively close to the coast, but avoiding the thick kelp.

Three kinds of acoustic stimuli were prepared on magnetic tape—a natural sequence of "screams" from killer whales, originally record-

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ed off Dabob Bay, Wash.; a matching sequence of two simultaneous pure tones of 500 and 2000 Hz that resembled the major frequency components in most of the recorded killer whale "screams" and with the same on-off times as the "screams"; and a similarly timed sequence of random noise in the band from 500 to 2000 Hz. Random noise and pure tone were used as control stimuli in addition to a third control wherein there was no playback or any intended stimulus, and the whales were allowed to pass relatively undisturbed. The ship's equipment was silenced throughout the experiments and we made no unnecessary noises.

The sound projector (designed and built at our laboratory) was lowered to 12 m below the surface of the water. It was powered with a 250-w amplifier (Optimation)[2] connected to the tape recorder (Uher 4200) used for playback. We monitored the output from a receiving hydrophone (Wilcoxon, Type M-H90-A) located at the same depth as the projector, 12 m away. Calderon and Wenz (1967) have described this calibrated monitoring system. Underwater signals picked up by the receiving hydrophone led to one track of another Uher stereo tape recorder. The other track carried our running commentary of the whales' behavior. Peak source level of the killer whale signals (Figure 1) and the playback control stimuli was nearly constant—151 db re 1 μNewton/m^2 (= 51 db re 1 dyne/cm^2) at 1 m in the 1969 experiments, and 176 db in 1970. Because of the natural propagation losses measured in the area, we expected sound pressure levels of the projected sounds to reach the prevailing ambient sea noise level in the third-octave band at 500 Hz, at about 1100 to 1400 m.

[2] The use of trade names is merely to facilitate description; no endorsement is implied.

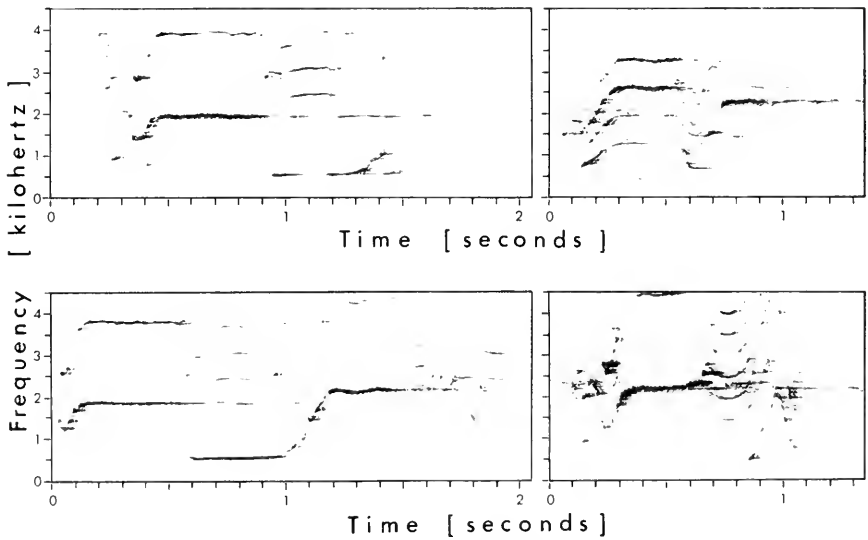

FIGURE 1.—Spectrograms of killer whale "screams" that were played back to migrating gray whales. Of 141 signals on the playback, 83 were very similar to the one at top, left. The others shown are in clockwise order according to their frequency of occurrence on the tape (30, 19, 9). The analyzing filter bandwidth was 20 Hz.

Besides no playback, used in the 1969 experiments, random noise and pure tone served as additional controls in 1970.

Gray whales come by Point Loma at any time during the migration season, day or night, as individuals or in small groups of two to five animals. However, for the total of 5 weeks at sea, we had to work in bright daylight, 0830 to 1630 hr, because of earlier difficulty in seeing the whales in subdued light. Undisturbed whales moved along in a southerly course at average speeds of 10 km/hr, blowing from one to three times every 1 to 14 min. We have also seen killer whales off Point Loma.

Migrating gray whales produce underwater, low-frequency moaning sounds in a band from 20 to 150 Hz (Cummings, Thompson, and Cook, 1968). These sounds last 1.0 to 1.7 sec.

We began an experiment upon seeing an oncoming whale or group of whales that was not encumbered with small boat traffic. Playbacks were generally started when the whales were 150 to 450 m away, toward the north. The experiments were alternated so that successive contacts would not encounter the same situation. Sessions lasted 30 to 100 min, after which the whales had passed the ship or disappeared. There were 77 experiments (77 contacts) involving a total of 132 gray whales. The appearance of "whale-watching" or fishing boats invalidated our work with numerous other contacts. They not only interfered with the whales' progress, but their underwater sounds considerably reduced the playback signal-to-noise ratio.

EXPERIMENTAL RESULTS

The reactions of gray whales to the projected killer whale sounds were spectacular. Blowing whales, or those running at the surface, immediately swirled around and headed directly away from the killer whale sound source. We have no idea of how quickly the submerged whales reacted, but subsequent surfacings were away from the killer whale sound source.

Most whales fled towards the north. However, if their previous southerly course had taken them seaward of the ship and the sound source, they turned and swam toward the open sea. Gray whales coming from the general direction of the kelp, or those very close to it, fled into this heavy growth and stayed in one general location until we stopped the killer whale stimulus.

Whales came about and resumed a southerly course for Mexico 5 to 30 min after the killer whale sound stopped. If we renewed the playback, before the whales passed by, they again fled northward from the sound source. On several occasions, we repeated the experiments on the same contact up to three times, each with similar results.

Of the 36 contacts principally involving killer whale playback, 30 avoided the sound source, 3 were questionable because we never saw them after playback, and 3 passed the ship as normal, without an apparent avoidance (Table 1). However, one of the last-mentioned was a single whale that had already come very close to the east-west line arbitrarily set on the projecting transducer. This whale could have just as easily avoided the sound source by swimming to the south.

TABLE 1.—Reaction of gray whales contacted on 77 occasions to various stimuli.
[1-4 whales in a contact; 132 whales in all.]

Reaction	Random noise	Pure tones	No playback	Killer whale "screams"
Avoidance	2	2	0	30
No avoidance	8	8	21	3
Questionable	0	0	0	3

All 21 contacts not encountering an intended stimulus (no playback) moved along towards the south in normal fashion. Two contacts of the 10 that received pure tone avoided the sound source. However, 1 of these 2 turned only momentarily. It then resumed the southerly course, still in the presence of the playback stimulus. Of the 10 contacts presented with random noise, 2 avoided by turning towards the north for a very short distance. However, both of these gray whales seemed to be startled only for the moment; then quickly turned a second time and swam down past the random noise sound source.

Two of the 8 contacts that did not avoid random noise and 2 of the 8 that did not avoid

pure tone (Table 1) did flee the killer whale source when we projected it to them before they passed by the sound projector. We did not project killer whale sound to the other contacts that did not avoid random noise and pure tone.

Observers on board noted how little of the avoiding whales' bodies showed above the surface and their unusually small surface disturbance. In many instances their blows were invisible and even blows at close range were scarcely audible. In contrast, the surfacing of undisturbed whales involved the simultaneous appearance of head and blow accompanied by a well-defined surface wake. Their blows were generally visible, and they were audible at close range. After their first appearance, the undisturbed whales showed their backs and sometimes tossed their flukes high into the air. On the other hand, it was difficult to spot the fleeing whales.

We thought that if gray whales associated the projected killer whale "screams" with potential danger, they would have left the area silently to lessen their chance of detection. For example, Schevill (1964) noted that belugas, *Delphinapterus leucas*, became silent in the vicinity of two killer whales. Upon analyzing our data, it turned out that only 2 gray whale phonations appeared on the tapes during killer whale periods, whereas 47 occurred during the control periods.

Gray whales on the breeding grounds frequently exhibit a behavior termed "spying-out" (Gilmore, 1961) wherein the head comes vertically out of the water for several seconds. "Spying-out" in the breeding lagoons seems to be associated with searching for channels; it is also done when the whales are pressed by small boats (communication from Dr. Joseph R. Jehl, San Diego Natural History Museum). Since we have seen this behavior over the past 6 years only three times, we assume that migrating gray whales rarely "spy-out." However, gray whales in the present experiments inevitably "spied-out" after fleeing into the thick kelp following killer whale playbacks.

As a result of these experiments we conclude that gray whales apparently recognize the voice of a killer whale, that they can easily localize the sounds underwater, and that they flee killer whale phonations probably as a sign of potential danger. Such avoidances consist of several behaviors that appear to function as protective mechanisms—sound localization, silence, rapid escape, reduced exposure, and visual search.

DISCUSSION

Walker (1971) has expressed the opinion that the function of "spy-hopping" ("spying out") by gray whales is not that ". . . the whale looks around, spying out possible dangers such as ships, or even spotting shore landmarks as aids to navigation." In reply he points out that "In the vertical posture the law of gravity takes over, conveying food from the whale's mouth to a capacious four-chambered stomach. Although a gray whale can swallow when horizontal, the vertical position allows it to clean entangled debris from the filter and to wash food down to the throat for quick ingestion." Walker further reported ". . . that in the 'spy-hopping' position their [gray whales'] range of vision is limited and that they navigate mostly by echolocation, ..." On the other hand, in the same report is the following portion of a caption to a photograph showing a breaching gray whale. "The catapulting action, he [Dr. Walker] believes, enables the animals to scan the waters around them and make course corrections when interfering noises prevent them from navigating by echo-location."

We are not aware of any experimental evidence that shows whether a gray whale "spy-hops" to look around or to swallow food. Nor is there any substantial evidence that migrating or breeding gray whales feed very much. To the contrary, there is hard evidence (summarized by Gilmore, 1968) that gray whales fast on their way to the breeding grounds, at the grounds, and on the return trip north.

Relative to the killer whale attack cited earlier, Baldridge reported that the mother frequently "spy-hopped" from a distance as the killer whale chewed on her dead calf.

In view of the above, the context of "spy-hopping" exhibited by gray whales during our experiments would seem to imply that this be-

havior had a visual function, i.e., they were probably looking for killer whales. This implication does not necessarily exclude swallowing, but we doubt that the gray whales were feeding under these circumstances.

Moreover, concerning Walker's assertion that gray whales "navigate mostly by echolocation," although it has been clearly demonstrated that porpoises can echolocate underwater objects (reviewed by Norris, 1969), it is not known whether they normally navigate at sea by this means. "Echolocation-like" (Asa-Dorian and Perkins, 1967) and "echolocation" sounds (Poulter, 1968) were recorded in the presence of gray whales, but there was little evidence that the sounds actually were from the whales. There are innumerable sounds in the ocean and many possible sources, but the matching correlations are often difficult to achieve. In any event, we prefer not to use the term "echolocation" in describing the underwater sounds of animals unless they are known to have such a function. Although we have recorded a few clicklike sounds during five seasons of work with gray whales, we have never been able to associate them with the whales. The clicklike sounds that were recorded, while using an array of hydrophones and a technique of computing sound source locations, originated from an area where, apparently, there were no gray whales.

Bioacousticians are not in a position to say with any certainty that gray whales do not echolocate; future experimental work could possibly show that they do. However, our own research with 7 of the 10 species of mysticate whales has yet to reveal any data to suggest that a member of this order uses underwater echolocation.

Whales normally exhale at the surface of the water with the blowholes exposed, but Hubbs (1965) has reported underwater blows by four species of whales—humpback, *Megaptera novaeangliae*; fin, *Balaenoptera physalus*; striped dolphin, *Lagenorhynchus obliquidens;* and the gray whale. In addition, he noted gray whales to refrain from spouting, skip an inhalation, or barely protrude their blowholes in the presence of killer whales. In the same report, Hubbs associated these behaviors with "strange or frightening stimuli." We were not close enough to see if fleeing gray whales exhaled underwater, but our other cited observations of gray whales covertly avoiding the killer whale sounds certainly parallel Hubbs' observations.

Unfortunately, there was no record of the killer whales' behavior during the original recordings of the phonations used for playback. We advise others who may be involved with sound playback to use appropriately meaningful signals, whenever possible. For example, underwater sounds that are part of an animal's reproductive behavior may have little or no effect on nonbreeding animals. Likewise, feeding sounds may not affect animals that are in a state of alarm. If possible, we would have compared the results of playing back two sets of killer whale sounds in the present experiments —one recorded from attacking whales in a predator-prey situation, if indeed killer whales utter sounds at this time, and another from killer whales that apparently were not feeding or preying.

Based on the methods of this study, our coworkers, Dr. James F. Fish, and John S. Vania subsequently used killer whale sound playback to keep white whales, *Delphinapterus leucas*, from entering the Kvichak River, Alaska, where they eat young salmon before the fish can get to the open sea (Fish and Vania, 1971).

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KILLER WHALE, *Orcinus orca*, SOUNDS REPEL WHITE WHALES, *Delphinapterus leucas*

JAMES F. FISH[1] AND JOHN S. VANIA[2]

ABSTRACT

This study was conducted to determine if the migration of white whales up the Kvichak River, Bristol Bay, Alaska, could be stopped by playing high-intensity underwater sounds to them. While in the river the whales feed on salmon smolt migrating down to the sea. Transmission of killer whale sounds was found to be an effective means for keeping the whales out of the river. During control periods when sound was not projected, the whales moved freely in and out of the river. A permanent playback system could be installed with little difficulty and would result in a significant reduction in the number of smolts consumed by belugas in the Kvichak River.

White whales, or belugas, *Delphinapterus leucas*, commonly travel 20 to 30 km up Alaska's Kvichak River (Figure 1) on the flood tide and back down on the ebb, foraging on available food organisms along the way. This twice-daily movement of from 50 to over 500 whales occurs during May and throughout most of June. It ceases only after boat traffic from the seasonal salmon fishery becomes heavy.

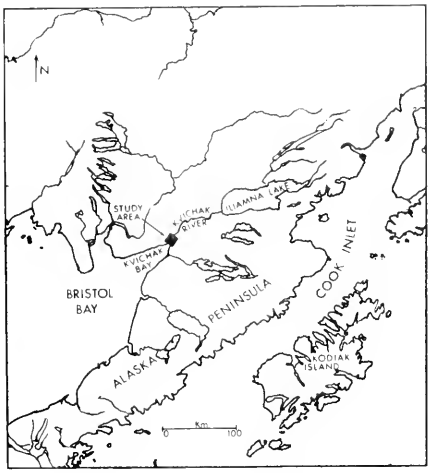

FIGURE 1.—Location of the study area.

The Kvichak River supports the most extensive run of red salmon, *Oncorhynchus nerka*, in the world. These runs range from 1 to 45 million fish per year. Lake Iliamna, the largest lake in Alaska, located about 80 km up the Kvichak, is the principal rearing area for the young salmon before they migrate to the sea as 1- or 2-year olds. The annual migration of smolt occurs during the end of May and the first 2 weeks in June with the peak of migration occurring about the first of June.

Field studies by the Alaska Department of Fisheries (1956) in the early 1950's showed that beluga predation on the salmon smolt occurred when the young fish were migrating to sea and appeared to be most extensive in the confines of the river. As the smolt moved into Kvichak Bay, they scattered and became less vulnerable to predation. Attempts were made to keep the belugas out of the river by chasing them with motorboats and by dropping small charges of explosives into the river. These methods were not very successful and were difficult to use during inclement weather or at night.

From 1963 to 1968 Vania projected various sounds, including killer whale sounds, noise, and music, underwater to the belugas in an attempt

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to keep them out of the river. There was no reaction to the noise or music. The killer whale sounds were only partially successful, apparently because the playback level was too low. His equipment was not designed for underwater sound transmission. Most other workers attempting to influence the movement of wild whales with sound have been unsuccessful. However, in nearly all of these experiments the projected sounds did not exceed 140 to 150 db, re 1 μNewton/m^2 (= 40 to 50 db, re 1 dyne/cm^2) at 1 m. One exception was a sound playback experiment on California gray whales, *Eschrichtius robustus*, by Cummings and Thompson (this issue of *Fishery Bulletin*) where a high-power transmitting system was used and the whales did react to the sounds. Their transmitting system was similar to the high-power system described here which we used to transmit killer whale sounds to belugas in the Kvichak River in June 1970.

METHODS

The primary high-power transmitting system, operated from Station A (Figure 2), consisted of a Uher tape recorder,[3] a small impedance-matching preamplifier, a 250-w Optimation power amplifier, and a sound projector developed and built by the Naval Undersea Research and Development Center. Frequency response of this system was ±3 db from 250 to 4000 Hz, limited by the projector. A secondary, battery-operated playback system, operated from a small boat at Station B (Figure 2), utilized a Uher tape recorder, a 40-w Bogen amplifier powered by a motorcycle battery, and a J-9 sound projector; system response was ±3 db from 200 to 6000 Hz.

A calibrated recording system, consisting of a Wilcoxon hydrophone, a Uher tape recorder, a sonar calibration box, and a modified GR Octave-Band Analyzer, was used to measure the sound pressure level of the playback signals at various points throughout the river and to record the vocalizations from the belugas. This system was described by Calderon and Wenz (1967).

[3] The use of trade names is merely to facilitate descriptions; no endorsement is implied.

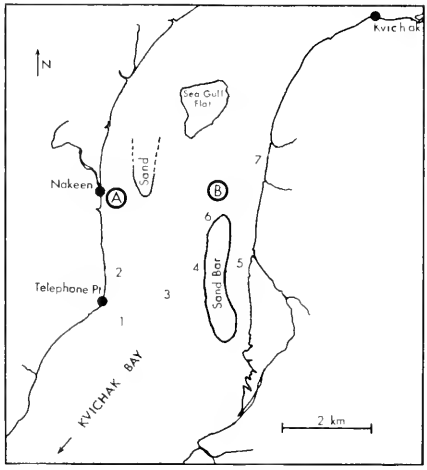

FIGURE 2.—Enlarged drawing of the study area. Transmitting stations were located at A and B. The regions indicated by numerals are locations where playback sound pressure levels were measured.

We selected killer whale, *Orcinus orca*, vocalizations for transmission because of their outstanding acoustic properties and because killer whales are known to kill and eat the relatively slow-swimming white whales (Scammon, 1874: 92; Dergerböl and Nielsen, 1930; Kleinenberg et al., 1964: 292). A long-play tape was made from "screams" and clicks recorded from killer whales at sea. We do not know, however, what behavior was associated with their sound production.

In the first 10 playback trials, the sound, transmitted only from Station A, was not turned on until the approaching belugas were sighted. But in subsequent trials, when we transmitted from both A and B, we started the playback as soon as the tide changed. Weather and light permitting, a new trial started with each flood tide and lasted from 15 min to 2 hr—as long as necessary to either move the whales back down the river (in those trials where they were permitted to move part way up before the sound

was turned on) or keep them from moving up the river (when the sound was turned on before the whales started upriver).

We had an excellent view of the river from the third floor of a building about 25 m above the water at Station A. Frequent low-altitude flights in small planes provided a check on the exact location of the whales when they could not be seen from the building. The white-colored whales were easy to see in the muddy brown river.

In all, there were 14 playback trials plus 7 control trials in which the whales were observed but sounds were not transmitted. The number of whales involved in each trial ranged from 50 to near 500 with about 100 being the average group size. Because several years of observations indicated the belugas move up the river on every incoming tide, we did not feel it was necessary to have an equal number of transmission and control trials. Consequently, we introduced a control after no more than four successive transmissions, primarily to determine if the belugas had stopped using the river as a result of the sound playbacks.

The sound pressure level of the playback sounds transmitted from Station A was measured at 6 locations in the river within 4 km of the source (Table 1). All levels reported in this paper are in db, re 1 $\mu N/m^2$. Sandbars prevented good transmission across the river, particularly during the early stages of the flood tide when some of the bars were covered by less than 0.5 m of water. For example, the sounds projected from Station A were below the background noise level at Location 6 (Figure 2). The best transmitting channel was downriver toward Telephone Point (Location 2), where a level of 132 db was measured for the playback signals. Beyond this area, the signal level dropped off quickly because of the shallow water which extended out a considerable distance from the Point. Most of the energy of the playback sounds occurred in a frequency band from 500 to 5000 Hz; thus we measured ambient levels in this band in addition to making broadband ambient measurements.

TABLE 1.—Sound pressure level (db re 1 $\mu N/m^2$) of playback signals and ambient noise at the indicated locations.

Location, Fig. 2	Signal broadband	Ambient noise	
		Broadband	500-5000 Hz band
	db	*db*	*db*
1 m from Source A	170	120	No data
1	115	103	96
2	132	90	84
3	107	91	85
4	103	95	90
6	Below ambient	98	95

RESULTS OF PLAYBACK EXPERIMENTS

In the first seven transmission trials, we let the whales move up the river on the west side to within approximately 2 km of the sound source before turning on the playback. The first six times, the animals turned immediately when the sound began and swam directly out of the river against the strong incoming tide. Their rate of blowing increased and they spent more time at the surface of the water, making it very easy for us to observe their course. On the 7th trial, some of the animals crossed to the opposite side of the river when the sound started and swam downstream along the sandbar (Location 4). The others remained just off Telephone Point for an hour, then moved out of the river.

It was difficult to see the approaching belugas on the 8th trial because of low light, and they had already moved up to Location 6 before we started transmitting. About half of the 100 whales continued up the river; the others turned and swam back out along the sandbar. On the next trial, the animals were first seen moving up the river along the sandbar. Possibly they learned from the previous eight transmissions to avoid the side of the river where the sound projector was located. They continued up to the end of the bar, even after the sound was turned on, but then rounded it and moved back down the river on the other side of the bar (Location 5).

The belugas similarly rounded the upriver end of the sandbar on the 10th trial; but, instead of turning around, they swam to the shore (Location 7) and continued up the river very close to the bank. Poor transmission to this location

from Station A and the reaction of the whales during the 10th trial made it necessary to use a second transducer simultaneously at Station B. A copy of the Station A playback tape was used at Station B, but it was not possible to synchronize the playback sounds from the two projectors. In the subsequent four trials, we turned on the playback as soon as the tide changed, thus eliminating the possibility of the whales going by us unnoticed before the start of transmission. The whales stayed at least 1.5 km down the river from the sound sources during the four transmissions using both projectors.

In each of the seven control trials, when no sound was played, the whales moved directly up the river with the incoming tide, past the transmitting stations. They behaved the same as they did before any of our experiments, with many individuals passing near the pier at Station A. However, we could see very few whales from the viewing station during the killer whale playbacks. Low-altitude reconnaissance flights confirmed our suspicion that numerous whales were remaining well down the river out of view during the transmissions.

Belugas vocalize extensively (Schevill and Lawrence, 1949; Fish and Mowbray, 1962). We recorded their sounds many times at various places throughout the river, often under ideal conditions of flat-calm water with no boat traffic. Once, we drifted in a small boat for over 2 hr during a no-playback control with a group of about 500 whales and recorded a spectacular variety of vocalizations from this relatively undisturbed herd. The belugas emitted very few sounds, however, when the killer whale signals were being transmitted. Quieting of belugas in the presence of killer whales was noted by Schevill (1964).

CONCLUSIONS AND RECOMMENDATIONS

Our experiments showed that playback sounds of killer whales can be used to keep belugas out of Alaska's Kvichak River. This method was very effective and practical. Installing and maintaining such a playback system for 2 to 3 weeks each year would seem to be an economically feasible way of reducing beluga predation on red salmon smolts. Such a system could be started automatically at the beginning of the flood tides and, if necessary, left on for their duration, both day and night. It would not be seriously affected by adverse weather conditions, except for a possible reduction in the range over which the belugas could hear the sounds—because of higher ambient noise levels in the river from wind and rough water.

We recommend using two sound projectors, properly situated to provide good signal levels all the way across the river. Initially, our trials with one projector stopped the whales, but the belugas eventually learned to go up the opposite side of the river where the playback signals were very weak or nonexistent. There were too many sand bars in the river to achieve good signal levels across the river with one projector, regardless of its source level.

Although we only used the two-projector system for four trials, we do not feel the whales would habituate and ignore the playback sounds with continued use. The avoidance of the belugas to this system was striking. The fact that the whales began swimming up the river again after nine trials using the single projector was due, we feel, to their learning to avoid the source, rather than to habituating to the sounds. With sound projectors located on both sides of the river there is probably no channel where the whales can go up the river without hearing the playback sounds. On the other hand, even if the belugas were to habituate after 2 weeks of playback, this would not significantly affect the usefulness of the technique. Playback could be timed to coincide with the 2-week peak of the smolt run.

After completing this experiment with killer whale playbacks, we projected a 2500-Hz continuous tone and 2500-Hz randomly pulsed tones. The belugas continued up the river during the continuous-tone playback, but turned back on the two occasions when pulsed tones were transmitted. Since these playbacks were tried after the white whales had been subjected to the killer whale sounds for 2 weeks, we cannot speculate on how naive whales would have reacted.

More experiments are necessary before we can conclude whether or not the belugas recognized the killer whale sounds as such.

ACKNOWLEDGMENTS

We thank Dr. William C. Cummings for his suggestions on the manuscript, George E. Lingle and John B. Webster for assisting with the data, and the New England Fish Company for providing laboratory space and aiding with the field work. Fish's work was supported by Independent Research funding of the Naval Undersea Research and Development Center during his appointment as a NRC-NUC Postdoctoral Research Associate, and Vania's in part by Federal Aid to Wildlife Restoration Funds, Project W-17-R.

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DEVELOPMENTAL ABNORMALITIES OF THE FLATFISH *Achirus lineatus* REARED IN THE LABORATORY[1]

EDWARD D. HOUDE[2]

ABSTRACT

Of 31 *Achirus lineatus* juveniles reared in a single experiment, 26 were abnormal. Abnormalities included incomplete eye migration, hooked dorsal fins, the presence of a left pectoral fin, ambicoloration, and partial albinism. The abnormal specimens have been described and photographed. A single reversed specimen, preserved as a nearly metamorphosed individual, also is described. Most of the abnormal conditions were interrelated. Possible effects of the rearing tank environment on abnormal development are discussed.

Larvae of lined soles, *Achirus lineatus* (Linnaeus), were reared in the laboratory from fertilized eggs collected in plankton tows from Biscayne Bay, Fla. Development and details of metamorphosis were described by Houde, Futch, and Detwyler (1970). No developmental abnormalities were detected until metamorphosis was nearly complete. At 50 days after hatching, the 31 survivors were examined and 26 (84%) were found to be abnormal.

The unique metamorphosis of flatfishes (Pleuronectiformes) may be responsible for the high percentage of abnormal specimens reported for this group in the literature (Norman, 1934; Hubbs and Hubbs, 1945; Dawson, 1962). Most of the described abnormal conditions in flatfishes were encountered in lined soles from this rearing experiment. Abnormalities included, in order of frequency, (1) ambicoloration (25 specimens), (2) retention of left pectoral fin, normally lost after metamorphosis (23 specimens), (3) partial or no migration of the left eye (17 specimens), (4) hooked dorsal fin, (same 17 specimens), (5) partial albinism (1 specimen), and (6) reversal (1 specimen). Similar abnormalities have been described previously in flatfishes, but are rare in the family Soleidae (Dawson, 1962). None have been reported previously for *A. lineatus*. The extremely high percentage of abnormal individuals among laboratory-reared specimens from this experiment apparently reflects some effect of the aquarium environment on the development of *A. lineatus*. A high proportion of pigment-deficient individuals of plaice (*Pleuronectes platessa*) was associated with high densities of metamorphosed specimens in rearing tanks and with low food levels in the tanks (Shelbourne, 1964, 1965; Riley, 1966), but other abnormalities were not discussed. Although the exact nature of the influence of the rearing tank on the production of abnormal specimens of lined soles is unknown, it is possible that harmful effects are created by (1) frequent contact with sides of small rearing tanks, (2) unnatural lighting, and (3) high concentrations of metabolites. Seshappa and Bhimachar (1955) reported failure of eye migration in the tongue sole *Cynoglossus semifasciatus* (Cynoglossidae) when postlarvae were kept in the dark. Two of my previous rearing experiments with *A. lineatus* also produced abnormal individuals. Some juvenile specimens of other flatfishes, *Paralichthys albigutta* (Bothidae) and *Gymnachirus melas* (Soleidae), also developed abnormally when reared from eggs hatched in my laboratory. The high incidence of abnormalities in the reared lined soles suggests that these conditions in flatfishes are not

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necessarily genetically controlled. When present in significant numbers, abnormalities may have important implications for those considering flatfishes in aquaculture.

METHODS

Rearing techniques were briefly described and larval development through metamorphosis reported by Houde et al. (1970). Methods used to rear *A. lineatus* were similar to those described in detail by Houde and Palko (1970). The 31 juveniles of lined sole were maintained in two 75-liter aquariums and fed on frozen brine shrimp (*Artemia salina*). Before metamorphosis larvae were fed wild zooplankton which consisted mostly of copepods. Beginning 50 days after hatching and at approximately 1-month intervals, growth was determined by measuring fish to the nearest millimeter total length (TL) and then returning them to the aquariums. Individuals were recorded as normal or abnormal when they were measured, the distinction being based on whether the dorsal fin was hooked.

Beginning 137 days after hatching, some fish were sacrificed and preserved for detailed examination. Radiographs were made to study skeletal structures. Specimens were accessioned into the fish collection at the Tropical Atlantic Biological Laboratory.

NORMAL SPECIMENS

Only five specimens were normal in all respects (Figure 1). Meristics and morphometrics of normal individuals fell within the range of variation for the species (Jordan and Evermann, 1898).

GROWTH AND MORTALITY

Growth was compared between grossly deformed specimens with hooked dorsal fins and normal specimens or those whose only anomalies consisted of unusual pigmentation and the presence of a left pectoral fin. Specimens with abnormal pigmentation or with left pectoral fins were classified as "normal" at the time they were measured because such conditions could not always be detected in small living juveniles. Although "normal" individuals initially were longer than abnormal ones, some compensation apparently occurred, and little difference in lengths was apparent between specimens still living from the two categories at 250 days after hatching. Both "normal" and abnormal specimens averaged about 52 mm TL at this time. No natural mortality occurred for either normal or abnormal lined soles between 50 and 275 days after hatching.

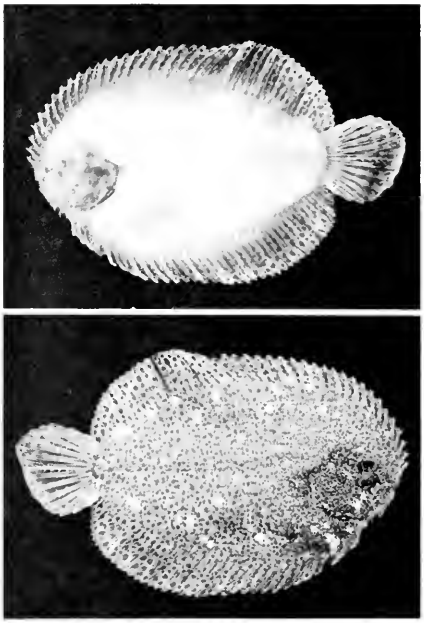

FIGURE 1.—Left side (upper photo) and right side (lower photo) of 45.8 mm TL normal *Achirus lineatus* reared in the laboratory. Dorsal fin was bent when specimen was preserved but it is normal.

TABLE 1.—Abnormalities of *Achirus lineatus* reared in the laboratory. A plus symbol indicates the presence of the abnormality and a minus symbol indicates its absence.

Specimen number	Total length	Pigment on blind side		Left pectoral fin	Hooked dorsal fin	Left eye migration
		Body	Head			
	mm	%	%			
64	32.6	100	100	+	+	incomplete
65	27.5	100	100	+	+	incomplete
68	29.5	100	100	+	+	incomplete
69	39.6	100	100	+	+	incomplete
72	45.8	100	100	+	+	incomplete
73	41.6	100	100	+	+	incomplete
84	42.7	100	100	+	+	incomplete
85	48.0	100	100	+	+	incomplete
88	60.0	100	100	+	+	incomplete
91	52.0	100	100	+	+	incomplete
92	50.0	100	100	+	+	incomplete
93	53.0	100	100	+	+	incomplete
67	34.8	100	100	+	+	nearly complete
83	43.8	100	100	+	+	nearly complete
87	61.0	100	100	+	+	nearly complete
90	77.0	100	100	+	+	nearly complete
75	58.4	100	100	+	−	complete
79	56.3	100	100	+	−	complete
89	69.0	100	100	+	−	complete
66	45.5	100	~70	+	−	complete
74	54.8	100	~70	+	−	complete
63	39.1	100	~10	+	−	complete
78	52.1	100	~10	+	−	complete
70	48.1	100	~10	−	−	complete
77	54.5	~90	0	−	−	complete
[1]86	66.0	0	0	−	+	no eye migration
71	45.8	0	0	−	−	complete
76	63.6	0	0	−	−	complete
80	42.6	0	0	−	−	complete
81	40.3	0	0	−	−	complete
82	52.9	0	0	−	−	complete

[1] This specimen had a small albinistic area on the right side of its head.

ABNORMALITIES

PIGMENTATION

Among the many abnormally pigmented specimens (Table 1) four major categories could be distinguished. Three were types of ambicoloration and the fourth was partial albinihm: (1) Nineteen specimens were completely pigmented on both sides (Figures 2 and 3). (2) Two specimens were completely pigmented except for the mouth region on the left (blind) side (Figure 5). (3) Four specimens were completely or almost completely pigmented on the body of the blind side but not on the head, which had little (10%) or no pigment (Figure 6). (4) One specimen was partially albinistic on the right side of the head, but other pigmentation was normal. The eye completely failed to migrate in this specimen (Figure 7). Normal coloration (five specimens) consisted of a lack of pigment on the blind side, except for tiny melanophores scattered over the posterior third of the body (Figure 1). All except two ambicolored individuals had some other associated abnormality, but normally pigmented specimens were always normal in other respects.

LEFT PECTORAL FIN

The left pectoral fin disappeared from normal lined soles when metamorphosis was nearly complete (Houde et al., 1970). A fin with 1 to 6 rays remained on 23 of the 31 juveniles from the rearing experiment. All specimens with a left pectoral fin also were abnormally pigmented.

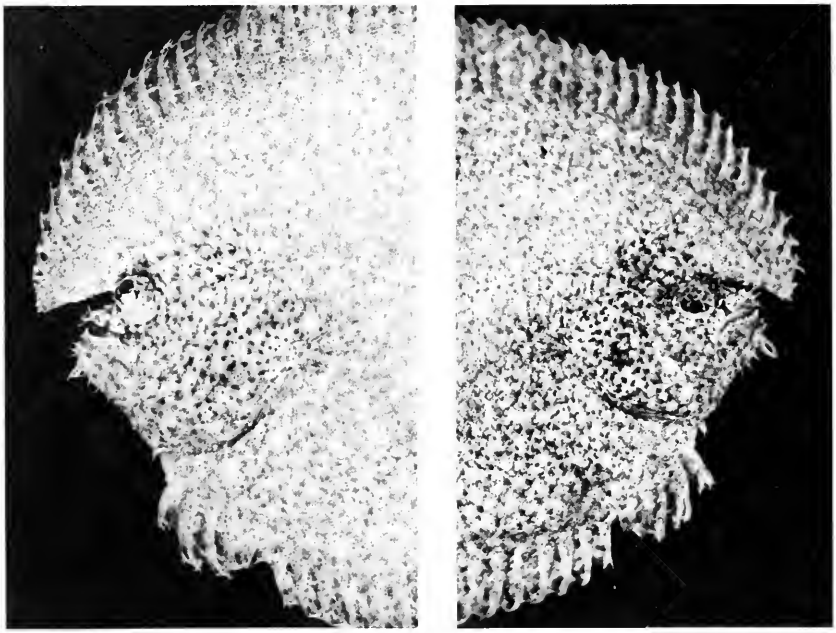

FIGURE 2.—Left side (left photo) and right side (right photo) of head of 39.6 mm TL abnormal *Achirus lineatus* reared in the laboratory. Ambicoloration, hooked dorsal fin, incomplete eye migration, and presence of a left pectoral fin.

Usually the fin was associated with a hooked dorsal fin and failure of eye migration (Figures 2 and 3), but a pectoral fin also was present on some individuals without those abnormalities (Figure 5).

HOOKED DORSAL FIN

Seventeen specimens had a hooked dorsal fin. In normal metamorphosis, the left eye of *A. lineatus* migrates across the dorsal midline under the projecting dorsal fin; the "hook" of the dorsal fin subsequently grows down toward the snout, eventually becoming adnate to the head. Development of the dorsal fin during metamorphosis was described for normal *A. lineatus* by Futch, Topp, and Houde.[3] In normal lined soles the first five dorsal fin pterygiophores articulate with serrations in the supraoccipital bone. The three anteriormost pterygiophores are directed anteriad, lying nearly parallel to the axis of the neurocranium. A fleshy connection is established between the dorsal fin and the prefrontal complex of the neurocranium. Radiographs of abnormal lined soles revealed that rotation of the frontal and prefrontal bones was incomplete during metamorphosis. The anteriormost pter-

[3] Futch, C. R., R. W. Topp, and E. D. Houde. Developmental osteology of the lined sole, *Achirus lineatus* (Pisces: Soleidae). (Unpublished manuscript.)

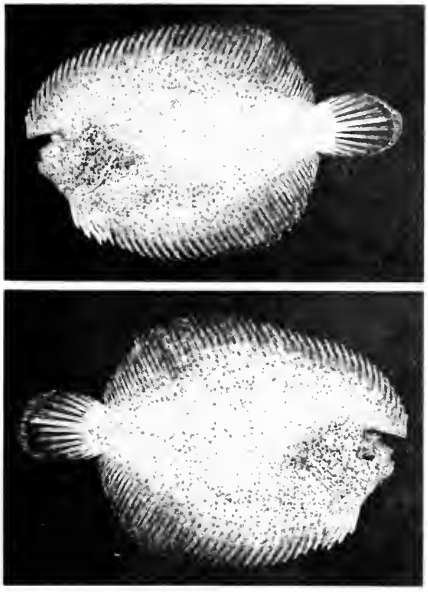

FIGURE 3.—Left side (upper photo) and right side (lower photo) of 32.6 mm TL abnormal *Achirus lineatus* reared in the laboratory. Abnormalities include ambicoloration, hooked dorsal fin, partial eye migration, and presence of a left pectoral fin.

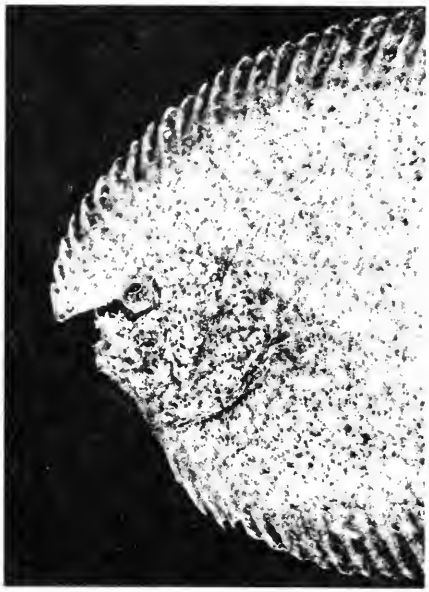

FIGURE 4.—Right side of head of 34.8 mm TL abnormal *Achirus lineatus* reared in the laboratory. Abnormalities include ambicoloration, hooked dorsal fin, and presence of a left pectoral fin. Eye migration nearly complete.

ygiophores appeared normal and articulated with the supraoccipital serrations, but were directed away from the neurocranium at an angle of about 20°. No fleshy connection was established between the dorsal fin and the incompletely rotated prefrontal complex. Hooked dorsal fins and eye migration failure are associated (Figures 2 and 3), but the hooked condition can also be present when eye migration is nearly normal (Figure 4).

EYE MIGRATION

Migration (rotation) of the left eye was incomplete in 17 specimens. The abnormality ranged from almost total failure of migration (Figures 2 and 7) to various stages of partial or nearly complete migration (Figures 3 and 4). All individuals with this abnormality also had hooked dorsal fins and were ambicolored or partially albinistic. In normal *A. lineatus* eye migration began at about 3.5 mm TL and was complete at 6.5 mm TL (Houde et al., 1970).

REVERSAL

A single reversed specimen was reared. This specimen was not included among the 31 treated in Table 1 because it was preserved before metamorphosis was completed. Reversals among the

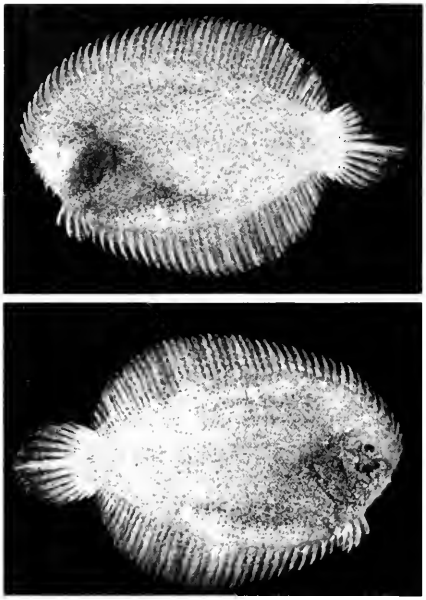

FIGURE 5.—Left side (upper photo) and right side (lower photo) of 45.5 mm TL abnormal *Achirus lineatus* reared in the laboratory. Abnormalities include ambicoloration and the presence of a left pectoral fin.

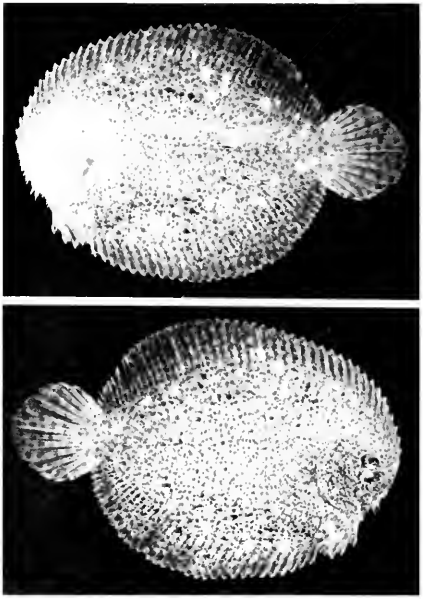

FIGURE 6.—Left side (upper photo) and right side (lower photo) of 48.1 mm TL abnormal *Achirus lineatus* reared in the laboratory. Specimen is ambicolored but otherwise normal.

Soleidae are extremely rare[1] (Hubbs and Hubbs, 1945). The specimen was a nearly metamorphosed individual of 5.5 mm TL (Figure 8), that appeared normal in other respects when compared with other postlarvae of the same length. Internal organs were not examined to determine whether they were reversed. Normal *A. lineatus* of the same length have been illustrated and described by Houde et al. (1970).

[1] The photograph in Herald (1961; fig. 139) should not be mistaken as a reversed *Gymnachirus williamsoni*, because in actuality this photograph was produced by an accidental reversal of a Kodachrome slide during preparation of the text.

SUMMARY

Most abnormalities of the laboratory-reared *A. lineatus* appeared related to each other. The presence of related anomalous conditions in individual specimens of flatfishes often has been reported, and the apparent association of ambicoloration, hooked dorsal fins, incomplete eye migration, and the tendency toward symmetry in paired fins has been discussed (Dawson, 1962; Gudger and Firth, 1936; Norman, 1934). The 26 aberrant juvenile specimens in my series of 31 were examined to determine the association of abnormal conditions in individual fish. Examination of Table 1 shows that the abnormalities are associated. Sixteen specimens had

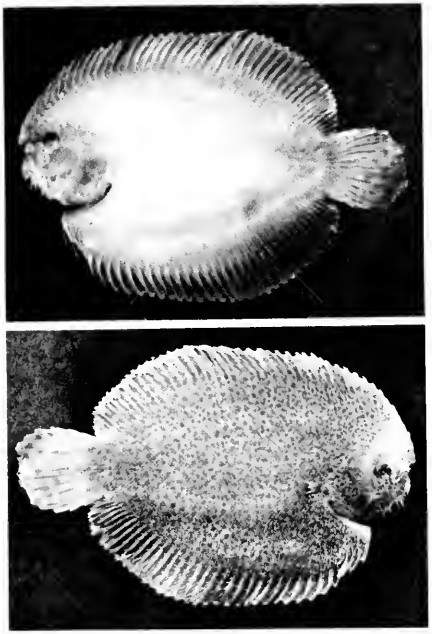

FIGURE 7.—Left side (upper photo) and right side (lower photo) of 66.0 mm TL abnormal *Achirus lineatus* reared in the laboratory. Partial albinism, hooked dorsal fin, no eye migration.

all four major anomalies and 16 to 23 specimens had two or three coexistent aberrancies. The "rule" of Gudger and Firth (1936), that was supported by extensive data on flatfishes (Dawson, 1962), stated that specimens with complete pigmentation of the body and pigmentation covering at least one-quarter to one-third of the head on the blind side will have a hooked dorsal fin and incomplete eye migration. Five of my lined soles fitted that category of ambicoloration but had neither a hooked dorsal fin nor incomplete eye migration (Table 1 and Figure 5). All but two ambicolored specimens also were abnormal in some other respect. Ambicolored individuals in which the body and more than 10% of the head on the blind side were pigmented retained a pectoral fin on the blind side. Eye migration never was complete in those specimens with a hooked dorsal fin.

A single specimen (Figure 7; #86 in Table 1) was unique in that the left eye completely failed to migrate. A portion of the right side of its head was unpigmented making it the only partially albinistic specimen in the series. The left side, which was unpigmented and lacked a pectoral fin, was similar in these respects to the blind side of normal lined soles. A well-developed hooked dorsal fin was present. Similar abnormalities were present in a naked sole (*Gymnachirus melas* Nichols) that was reared at the laboratory.

The high percentage of abnormalities in laboratory-reared *A. lineatus* must have been influenced by rearing conditions, since abnormal lined soles apparently are extremely rare in nature. Further controlled experiments should make it possible to determine what factors cause abnormal metamorphosis of lined soles and perhaps other flatfishes. These experiments also might test the common assumption that survival of abnormal flatfishes is lower than that of normally metamorphosed individuals, since no advantages in either survival or growth of normal juveniles of *A. lineatus* were detected in the initial rearing experiment.

ACKNOWLEDGMENTS

Assistance in rearing the larvae was provided by Barbara Palko and Robert Detwyler. Charles

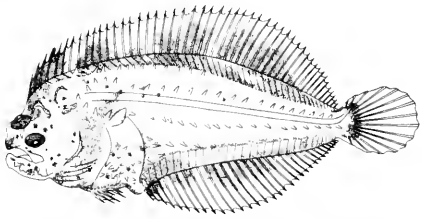

FIGURE 8.—Left side of a reversed, nearly metamorphosed, 5.5 mm TL specimen of *Achirus lineatus* reared in the laboratory.

Futch, William J. Richards, and C. R. Robins have reviewed and criticized the manuscript. The reversed specimen was illustrated by Grady Reinert. Photographs were taken by Andrew Ramsay and Anna Delor.

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THE EARLY LIFE HISTORY OF SKIPJACK TUNA, *Katsuwonus pelamis*, IN THE PACIFIC OCEAN

HOWARD O. YOSHIDA[1]

ABSTRACT

This study investigates the early life history of skipjack tuna, including the distribution, abundance, age, and growth. The study is based on 1,742 juvenile skipjack tuna that were found in the stomachs of 6,867 billfishes caught in Hawaiian waters and in the South Pacific by commercial longline boats. The smallest juvenile taken near Hawaii was 5.9 cm in standard length, and in the South Pacific 1.6 cm in standard length. Regressions describing the relations between the standard length and lengths of (1) the vertebral column, (2) the precaudal vertebrae, (3) caudal vertebrae, (4) the 1st-10th vertebrae, and (5) the 21st-30th vertebrae of juvenile skipjack tuna were determined. The regressions provided estimates of the standard length of fragmentary specimens. Juvenile skipjack tuna were widely distributed between lat 5° and 32° S, and long 137° W and the 180th meridian. North of the equator, the commercial longline boats fished close to the main Hawaiian Islands, and thus only a limited picture was obtained of the areal distribution of juvenile skipjack tuna. Juvenile skipjack tuna were found in almost all months in Hawaiian waters. They were most numerous in July and August. In the South Pacific, juveniles were also found in almost all months between lat 5° and 20° S. Peaks in the apparent abundance were evident in April and October in the area north of lat 10° S. Juvenile skipjack tuna appeared to be more numerous in the South Pacific than around Hawaii.

Length-frequency distributions of juvenile skipjack tuna from Hawaii showed well-defined modes, which progressed with time. The growth of the juveniles was estimated by using the modal lengths determined from the monthly length-frequency distributions. Skipjack tuna between 9 and 40 cm around Hawaii are estimated to grow 2.0 cm per month. One-year-old fish are estimated to be 31 cm in standard length.

In 1968, 70,746 metric tons of skipjack tuna, *Katsuwonus pelamis*, were landed in the eastern Pacific (Inter-American Tropical Tuna Commission, 1970) and 109,018 metric tons were landed in Japan (Japan. Fisheries Agency, Research Division, 1970). Because of its commercial importance much knowledge has been accumulated on the biology of the skipjack tuna. Information on early life history, however, is incomplete. Matsumoto (1958) described skipjack tuna larvae and their temporal and spatial distribution in the central Pacific. Ueyanagi (1969) reported on the distribution of larval skipjack tuna in the Pacific Ocean between 1960 and 1967, and Higgins (1967) summarized the distributional records of juvenile skipjack tuna in the Pacific.

The present study is based on immature skipjack tuna between 1.6 and 40 cm taken from the stomachs of billfishes near Hawaii and in the South Pacific. Included are observations on geographical and seasonal distribution, length-frequency distributions, and age and growth rates.

MATERIALS AND METHODS

The stomachs of 6,867 billfishes[2] were examined in this study. Those examined included 4,118 striped marlin, *Tetrapturus audax;* 1,606 blue marlin, *Makaira nigricans;* 383 shortbill spearfish, *T. angustirostris;* 216 sailfish, *Istiophorus platypterus;* 196 swordfish, *Xiphias gladius;* 171 black marlin, *M. indica;* and 177 billfishes that were not identified to species.

[1] National Marine Fisheries Service, Hawaii Area Fishery Research Center, Honolulu, Hawaii 96812.

[2] The term billfishes as used in this paper includes swordfish.

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Sixty-six percent of the stomachs came from billfishes captured near Hawaii between July 1962 and April 1966. These stomachs were used previously in an early life history study of albacore, *Thunnus alalunga* (Yoshida, 1968). Most of these billfishes were caught within 37 km (20 nautical miles) of the main islands. A few were caught as far as 740 km (400 nautical miles) from Oahu. Thirty-four percent of the stomachs came from billfishes caught in the South Pacific between lat 5° and 32° S, and between long 135° W and 179° E (Figure 1). These were caught on longline gear by boats operating out of American Samoa between January 1964 and July 1966. The fishery has been described by Otsu and Sumida (1968).

Arrangements were made with the crews of several boats to have billfish stomachs collected on the fishing voyages. Each cooperating crew was provided with a stainless steel tank, formaldehyde solution, labels, and collecting bags. The crew was paid 50 cents for each stomach.

In the laboratory at Honolulu, all tuna and tunalike specimens were sorted from the stomach contents and identified. Skipjack tuna were identified by skeletal characters (Godsil and Byers, 1944). Standard length was recorded for all intact specimens. Following a technique used earlier (Yoshida, 1968), a method was devised to estimate the standard length of fragmentary specimens. Relations were determined between the standard length and the length of: (1) the complete vertebral column (41 vertebrae), (2) the precaudal vertebrae (vertebrae 1-20), (3) the caudal vertebrae (vertebrae 21-41), (4) 1st-10th vertebrae, and (5) 21st-30th vertebrae, based on 77 intact juvenile skipjack tuna specimens from around Hawaii and the South Pacific. All the relations appeared to be linear and straight lines were fitted to the data by the method of least squares. Combining the samples from Hawaii and the South Pacific should not adversely affect the results. A plot of the data for all five relations did not indicate any differences between the North Pacific and South Pacific samples. A covariance analysis applied to the relation between the standard length and the length of the complete vertebral column for Hawaiian and South Pacific juvenile

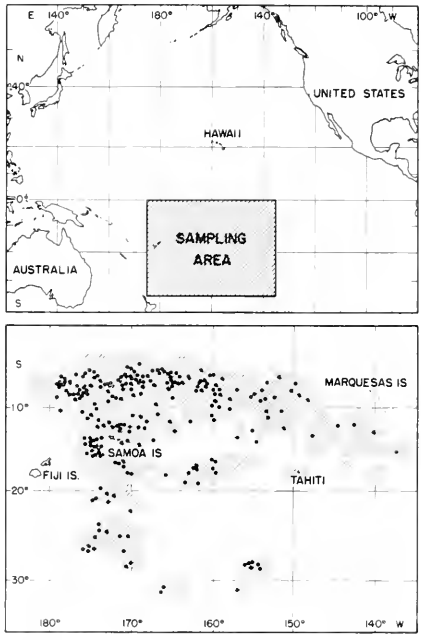

FIGURE 1.—The location of capture of billfishes (shaded area) and the distribution of juvenile skipjack tuna (dots) in the South Pacific.

skipjack tuna confirmed the lack of significant differences between the samples. No significant differences were found in the regression coefficients ($F = 0.105$; df = 1, 73) and in the intercepts ($F = 0.053$; df = 1, 74). The regressions of the standard length on the various vertebral segments are presented in Table 1.

The lengths of most of the specimens were estimated by using a suitable regression. For 22% of the specimens the relative position of the fragments could not be determined, and the regressions were not used. For these specimens the standard length was estimated by comparing the average length of the vertebrae in the fragment with the average length of the vertebrae

of the specimens used to calculate the regressions.

TABLE 1.—Regressions describing the relations between the standard length and lengths of the vertebral column, precaudal vertebrae, caudal vertebrae, 1st-10th vertebrae, and 21st-30th vertebrae of juvenile skipjack tuna [l = standard length (cm), L = length of vertebral fragments (cm)].

Segment of vertebral column	Regression	Standard deviation from regression
Complete vertebral column	$l = 0.0693 + 1.2262L$	0.435
Precaudal vertebrae	$l = 0.6544 + 2.4926L$	0.439
Caudal vertebrae	$l = -0.4414 + 2.4196L$	0.445
1st-10th vertebrae	$l = 0.4280 + 5.2938L$	0.515
21st-30th vertebrae	$l = -0.2637 + 4.6942L$	0.595

DISTRIBUTION AND ABUNDANCE

Seasonal and areal coverage was spotty, but sampling was extensive enough to permit meaningful analysis for the present study. In the following sections I will discuss the distribution and the seasonal and annual apparent abundance of juvenile skipjack tuna in the South Pacific and near Hawaii.

AREAL DISTRIBUTION

Commercial longline boats engaged in collecting billfish stomachs in the South Pacific ranged over a wide area, and juvenile skipjack tuna, as indicated by their presence in billfish stomachs, also were widespread (Figure 1). Around Hawaii fishing was restricted to a relatively small area, and so a more limited picture was obtained of the areal distribution of juvenile skipjack tuna.

Skipjack tuna larvae are widely distributed in the Pacific Ocean (Matsumoto, 1966; Ueyanagi, 1969). Ueyanagi (1969) reports that larvae were taken across the entire South Pacific between the equator and lat 10° S. Also, west of long 140° W larvae were taken as far south as lat 32° S. My study shows that the distribution of juvenile skipjack tuna is similar to the distribution of the larvae. In the North Pacific, skipjack tuna larvae have been found around Hawaii and across the entire Pacific between the equator and lat 20° N. In the western North Pacific, between long 160° W and the Asian continent, larvae have been taken almost as far north as lat 35° N (Ueyanagi, 1969).

My study indicates that gaps in the distribution of juvenile skipjack tuna in the Pacific reflect a lack of sampling. Higgins (1967) has a somewhat similar viewpoint. It is likely that the juveniles are as widely distributed as larval skipjack tuna in the North Pacific.

The distribution of juvenile skipjack tuna in the South Pacific by quarters of the year (all years combined) is shown in Figure 2. This is only the apparent distribution, however, because it reflects the operations of Samoa-based vessels. These vessels primarily seek albacore, and therefore they fish the areas where albacore catch rates tend to be high. In the first half of the year, vessels generally operate north of lat 20° S, and in June or July they move as far south as lat 30° S before heading north again (Otsu and Sumida, 1968). Samples were available mostly from north of lat 20° S, and juvenile skipjack tuna were found throughout the sampling range. In the third and fourth quarters, samples were available from a wider area, and again juvenile skipjack tuna were taken from almost the entire area sampled. Although seasonal coverage was incomplete throughout the total area, synoptic sampling would probably produce juveniles in all seasons and throughout the total area.

SEASONAL APPARENT ABUNDANCE

Hawaii

Apparent abundance is expressed here as number of skipjack tuna per 100 billfish stomachs. The apparent abundance of juveniles around Hawaii, all years combined, is shown in Figure 3. The juveniles were more numerous during July, August, and September. A peak in abundance usually occurred in August. These observations confirm Matsumoto's (1966) conclusion that skipjack tuna in the Hawaiian Islands spawned during the summer. He showed that the abundance of larval skipjack tuna peaked in July.

The apparent abundance of juveniles in 1963, 1964, and 1965 offers interesting contrasts. For example, in August 1963 the apparent abundance peaked sharply to 100 juveniles per 100 bill-

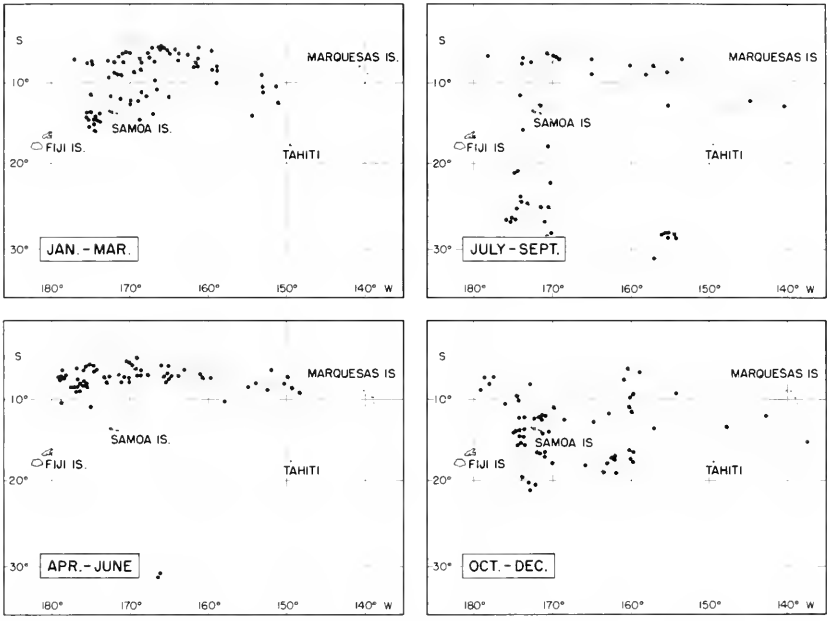

FIGURE 2.—Distribution of juvenile skipjack tuna (dots) by quarters of the year in the South Pacific. The shadings show the billfish sampling area.

fishes. Only small numbers were taken between January and June and between October and December. In 1964 and 1965 the summer peak in abundance was not so high; however, juveniles were more numerous in other months. The apparent abundance of juveniles was highest in 1964 when an average of 21.3 juveniles per 100 billfishes was taken. In 1965, an average of 19.1 juveniles was taken, and in 1963 the average was 12.4.

South Pacific

To examine the seasonal apparent abundance of juvenile skipjack tuna in the South Pacific, the area north of lat 10° S was considered separately from the area between lat 10° and 20° S (Figure 4). Because the coverage was poor in any one year, the data for all years were combined.

Juvenile skipjack tuna were taken throughout the year north of lat 10° S. Peaks in the apparent abundance were evident in April and October. Larvae have been taken throughout the year in the equatorial waters (lat 10° N to 10° S) of the central Pacific (Matsumoto, 1966). They were most numerous between April and July. Thus, the apparent abundance of the juveniles differs somewhat from that of the larvae in that a peak was absent in larval abundance in the latter half of the year. The difference in apparent abundance between the larvae and juveniles may not be real. The small number of stomachs collected in the latter half of the year may not have been adequate to reveal the true abundance of juveniles.

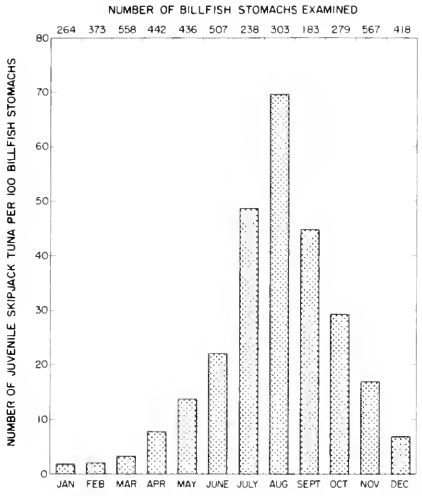

FIGURE 3.—Apparent abundance of juvenile skipjack tuna in Hawaiian waters, 1962-66.

Juveniles were taken in all months except July between lat 10° and 20° S. Here also the uneven sample sizes make the analysis of apparent abundance difficult. It appears, however, that juveniles were numerous from November to February. No comparable data on larval skipjack tuna in this area are available.

The apparent abundance of juvenile skipjack tuna was higher in 1964 than in 1965 in the South Pacific (Table 2). In 1964, juveniles were slightly more numerous north of lat 10° S than between lat 10° and 20° S. In 1965, however, juveniles were more numerous between lat 10° and 20° S than north of lat 10° S. Also they appeared to be more numerous in the South Pacific than near the Hawaiian Islands.

TABLE 2.—Annual apparent abundance of juvenile skipjack tuna in the central Pacific Ocean.

Year	Area	Number of billfishes	Number of juvenile skipjack tuna	Number of juveniles per 100 billfishes
1963	Hawaii	1,351	167	12.4
1964	Hawaii	1,608	342	21.3
	Equator-10° S	579	268	46.3
	10°-20° S	216	92	42.6
1965	Hawaii	1,008	193	19.1
	Equator-10° S	208	60	28.8
	10°-20° S	537	191	35.6

About 12% of the billfish stomachs from Hawaii contained one or more juvenile skipjack tuna, while 19% of the billfish stomachs from the South Pacific contained one or more juveniles. In both areas the largest number of juvenile skipjack tuna per stomach was 11; most of the stomachs from both areas had only one juvenile.

AGE AND GROWTH

The length-frequency distribution of juvenile skipjack tuna from Hawaii and the South Pacific is shown in Figure 5. Near Hawaii the smallest juvenile taken was 5.9 cm SL (standard

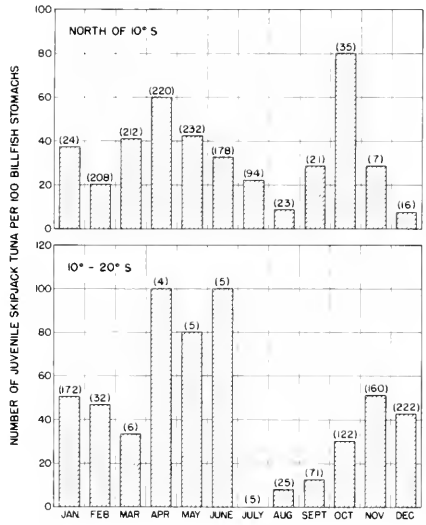

FIGURE 4.—Apparent abundance of juvenile skipjack tuna in the South Pacific. The figures in the parentheses are the number of billfish stomachs that were examined.

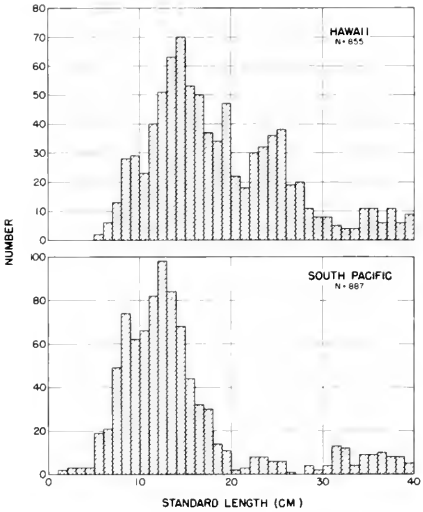

FIGURE 5.—Length-frequency distribution of juvenile skipjack tuna.

length). A prominent mode was located at the 14-14.9 cm size class, a lesser mode at 25-25.9 cm, and a relatively obscure mode was evident between 30 and 40 cm. The length frequencies of juvenile skipjack tuna from the South Pacific were similar to those from Hawaii. The smallest juvenile taken in the South Pacific was 1.6 cm SL. A prominent mode was evident at the 12-12.9 cm length class, a lesser mode between 20 and 30 cm, and a relatively obscure mode was apparent between 30 and 40 cm.

The monthly length-frequency distributions of the juveniles near Hawaii show well-defined modes in the summer and fall (Figure 6). Some of the length distributions have two or more well-defined modes. These modes probably represent progeny from two or more spawnings spaced more or less closely in time. An increase in size of the juveniles, as indicated by a progression of the modes, was evident in the monthly length distributions. In contrast, Higgins (1970) found that modal groups of juvenile skipjack tuna taken in midwater trawls did not show an increase in length with time.

Because the modal lengths increased with time, an attempt was made to describe the growth of the juveniles. To facilitate the designation of modes, the length data were smoothed by a moving average of three. Modes were not considered in samples that had fewer than 10 fish, nor in length groups containing fewer than 5 fish. The selected modes are given in Figure 7 and Table 3. Using the method of least squares, straight lines were fitted to groupings of modal points to represent the apparent growth of the juveniles. The slope of the lines for 1962, 1964-65, and 1965-66 indicates that juvenile skipjack tuna grow about 2.0 cm per month. The slope of the line for 1963-64 indicates a growth of 1.2 cm per month. These data suggest annual differences in the growth of the juveniles. I believe that an increment of 2 cm per month is the best estimate of the growth rate of juvenile skipjack tuna within the length range covered in this study. Rothschild (1967) used data from tag and recapture experiments and the von Bert-

TABLE 3.—Modes in the length-frequency distributions of juvenile skipjack tuna from Hawaii (from Figure 6).

Year	Month	Standard length (cm)		
1962	August	13.0	19.0	23.5
	September	20.0		
	October	27.0		
	November	22.0		
1963	June	13.5		
	July	13.5		
	August	9.5	15.5	
	September	15.5	22.0	
	November	23.5		
	December	24.0		
1964	April	30.0		
	May	19.0	25.0	
	June	15.5	25.5	
	July	8.5	13.5	
	August	11.0	18.5	
	September	12.5		
	October	14.5	23.5	
	November	23.5		
	December	25.0		
1965	April	35.5		
	May	12.5	26.5	
	June	9.5		
	July	15.5		
	August	10.5	14.5	18.5
	October	13.0	19.5	
	November	18.5		
1966	April	32.5		

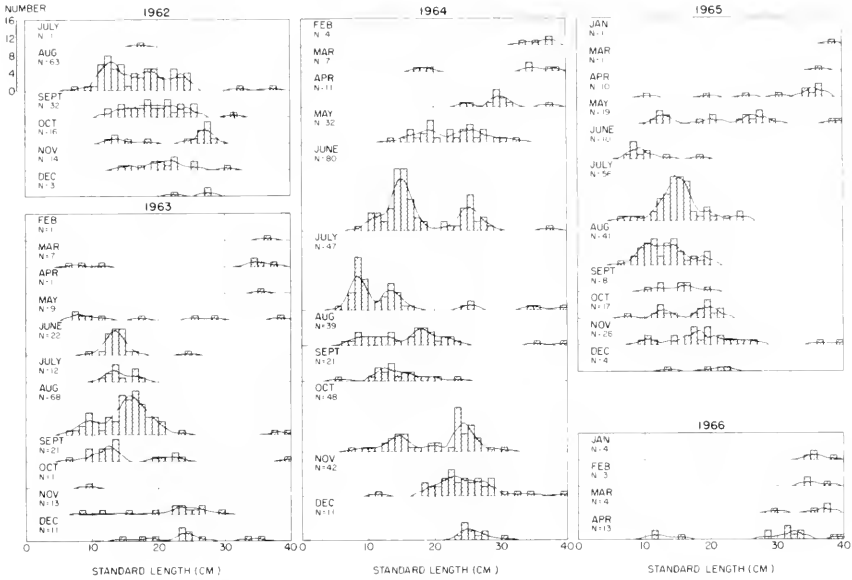

FIGURE 6.—Monthly length-frequency distribution of skipjack tuna from Hawaii.

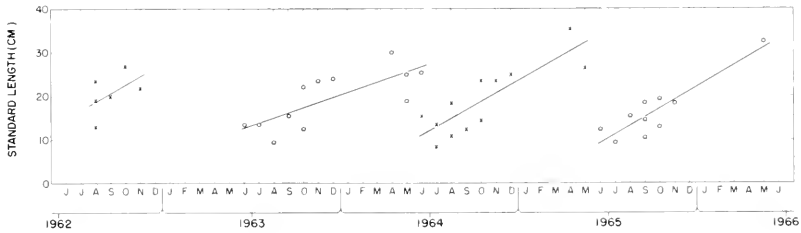

FIGURE 7.—Growth of juvenile skipjack tuna from Hawaii.

alanffy growth function to estimate skipjack tuna growth. He indicated that skipjack tuna between 44 and 72 cm grew about 16.6 cm per year or 1.4 cm per month. The deceleration in growth from 2.0 cm per month for skipjack tuna between 9 and 40 cm to 1.4 cm per month for fish between 44 and 72 cm is to be expected.

It is useful to determine the growth rate of skipjack tuna from hatching to 9 cm SL. These and other data may make it possible to estimate

the age of skipjack tuna at various sizes. The growth rate of a related species, black skipjack, *Euthynnus lineatus*, between 17 and 75 mm SL has been determined (Clemens, 1956). In experiments conducted in shipboard aquaria Clemens showed that black skipjack grew 36.8 mm in 295 hr, which would be about 9 cm in a month. Houde and Richards (1969) reared larval little tunny, *E. alletteratus*, under laboratory conditions and reported similar growth rates. It was recently found that larval skipjack tuna have similar growth rates (personal communication, William J. Richards, Supervisory Zoologist, NMFS Tropical Atlantic Biological Laboratory, Miami, Fla. 33149, August 18, 1970). If larval skipjack tuna grow at the same rate (9 cm per month) for the first month and 2 cm per month for the next 11 months, then skipjack tuna 1 year old would be 31 cm SL.

Brock (1954) assumed that the modal sizes of fish between 40 and 50 cm FL (fork length) that appear in the Hawaiian skipjack tuna fishery during the summer represented 1-year-old fish. Forty to fifty centimeters for 1-year-old fish seems too high. Modal lengths typical of winter skipjack tuna in the Hawaiian fishery are 35, 50, and 70 cm FL (Rothschild, 1965). My data indicate that the 35-cm modal group that appears in the winter represents 1-year-old fish.

SPAWNING

In the Hawaiian area, juvenile skipjack tuna smaller than 10 cm were found during 7 months in 1963 and during 5 months in 1964 and 1965 (Figure 6). This suggests a protracted spawning season and nearly continuous recruitment of juveniles. The catch of larvae indicates that spawning begins in March, peaks in July, and declines sharply in September and October (Matsumoto, 1966).

The Hawaiian skipjack tuna fishery peaks in the summer when the bulk of the catch is composed of "season-size" fish larger than 60 cm (Rothschild, 1965). Larval and juvenile skipjack tuna also are most numerous during the summer, which suggests that the large fish spawn in Hawaiian waters. The presence of juveniles in the spring and fall, although in lesser numbers, indicates that spawning also takes place then. Rothschild (1965) has hypothesized that at least one subpopulation spawns in Hawaiian waters. The protracted spawning season may indicate that more than one subpopulation spawns here.

In the South Pacific between lat 5° and 20° S, the spawning season is more protracted than in Hawaiian waters. Juveniles smaller than 10 cm SL were taken in almost every month north of lat 10° S. Between lat 10° and 20° S they were taken in all months except March, April, June, and July (Figure 8); the reason for their absence in these months may be inadequacy of sampling. The area south of lat 20° S was somewhat different. Although sampling was sparse, the length-frequency distribution consistently showed few small juveniles, which indicates little or no spawning in this area.

The length-frequency distribution of juvenile skipjack tuna north of lat 10° S and between lat 10° and 20° S indicated a progression of modes (Figure 8). However, a plot of the modal lengths indicated no growth. The cause of this may be a protracted spawning season and inadequacy of samples. Also juveniles appear to migrate southward as they grow. Only small numbers of juveniles larger than 20 cm SL were taken north of lat 20° S, and most of the juveniles taken south of lat 20° S were larger than 20 cm SL.

ACKNOWLEDGMENTS

Thanks are due the research assistants at the NMFS Hawaii Area Fishery Research Center who examined the billfish stomach contents for juvenile skipjack tuna and aided in summarizing part of the data. The portion of the study in the South Pacific would not have been possible without the cooperation of the crews of the many fishing vessels from Japan and the Republic of Korea. I thank William J. Richards, NMFS, Miami, Fla.; Walter T. Pereyra, NMFS, Seattle, Wash.; and Witold L. Klawe, Inter-American Tropical Tuna Commission, La Jolla, Calif. for reviewing the manuscript.

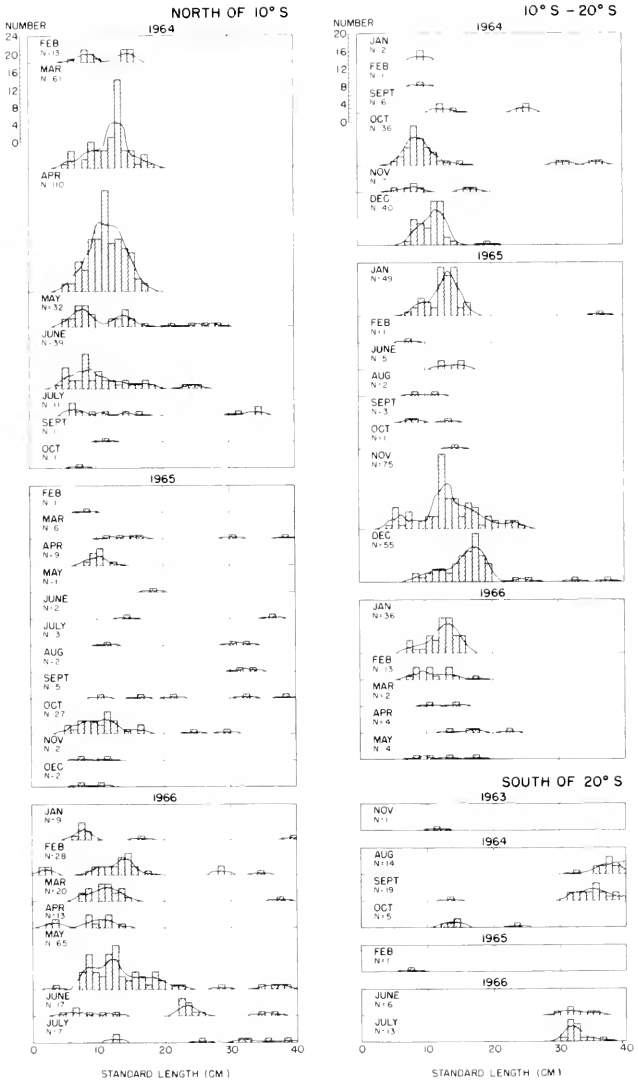

FIGURE 8.—Monthly length-frequency distribution of juvenile skipjack tuna north of lat 10° S, lat 10°-20° S, and south of lat 20° S in the South Pacific.

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DISTRIBUTION OF TUNA LARVAE (PISCES, SCOMBRIDAE) IN THE NORTHWESTERN GULF OF GUINEA AND OFF SIERRA LEONE[1]

WILLIAM J. RICHARDS AND DAVID C. SIMMONS[2]

ABSTRACT

Investigations of tuna larvae distributions in the northwestern Gulf of Guinea and off Sierra Leone were made during February-April 1964, August-October 1964, and February-April 1965. Larvae of the yellowfin tuna, bigeye tuna, skipjack tuna, little tunny, and frigate mackerels were collected and studied. Analyses of the data indicated that larvae of yellowfin tuna and bigeye tuna migrate to the surface during the day, skipjack tuna migrate to the surface during the night, and frigate mackerels do not seem to migrate at any time. Our data for little tunny were inconclusive. All species were widely distributed over the area but larvae of the commercially important tunas—yellowfin, bigeye, and skipjack—were restricted to waters where surface temperatures were higher than 24° C.

The distribution of tunas varies seasonally in the eastern Atlantic Ocean (Richards, 1969). In 1964 and 1965, the Bureau of Commercial Fisheries research vessel *Geronimo* (cruises 3, 4, and 5) collected tuna larvae in the northwestern Gulf of Guinea and off Sierra Leone. These collections were part of extensive investigations intended to relate the spatial and temporal distributions of tunas to the environment. Cruise 3 was in the northwestern Gulf of Guinea between 10 February and 26 April 1964, which is within the winter-spring "warm season" in the Gulf of Guinea, when sea-surface temperatures are higher than during summer and fall. Cruise 4 was in the northwestern Gulf of Guinea between 5 August and 13 October 1964, which is within the summer-fall "cool season" in the Gulf of Guinea, when sea-surface temperatures are lower than during winter and spring. During cruise 5, collections were made in two areas: the northwestern Gulf of Guinea and off Sierra Leone. The northwestern Gulf of Guinea area was generally the same as that covered in cruises 3 and 4 and collections were made from 14 March to 19 April 1965 within the winter-spring "warm season." The area off Sierra Leone, which is immediately northwest of the areas covered in cruises 3, 4, and part of 5, was studied from 10 February to 2 March 1965 (see Figure 1).

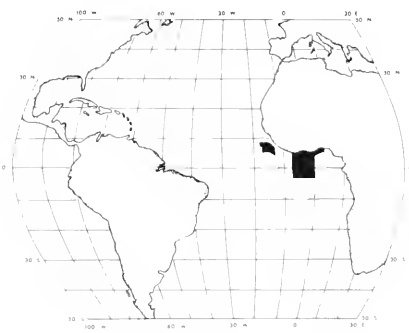

FIGURE 1.—Reference map for the areas studied. The shaded area east of long 10° W was surveyed on *Geronimo* cruises 3, 4, and part of 5; the shaded area west of long 10° W was surveyed on part of cruise 5.

The purposes of this study are to (1) analyze the time the collections were made, (2) describe the distribution of the tuna larvae, and (3) discuss the relations of the tuna larvae to oceanographic features. In addition to the collecting of larvae on each cruise, sightings of surface

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schools of tuna were recorded and several oceanographic features—temperature, salinity, and dissolved oxygen—were measured. The distributions of these oceanographic features were published in a series of atlases (Goulet and Ingham, 1968; Ingham, Goulet, and Brucks, 1968; Brucks, Ingham, and Leming, 1968a, 1968b).

The northwestern Gulf of Guinea is affected by the general meteorological and oceanic conditions of the Gulf of Guinea and by some unique local features. Ingham (1970) concluded that two types of upwelling occur in this region—a seasonal wind-driven upwelling (July through October) and a current-induced upwelling that is present most of the time. The mixed surface layer is rather thin in the coastal area (less than 10 m near the coast, grading to 30 to 40 m offshore) and is influenced by current-induced upwelling, wind-driven upwelling, and advection. Ingham (1970) reported that during the period of *Geronimo* cruises 3, 4, and 5, advection was the most effective of the three factors.

The species collected were the yellowfin tuna, *Thunnus albacares* (Bonnaterre); the bigeye tuna, *Thunnus obesus* Lowe; the bluefin tuna, *Thunnus thynnus* (Linnaeus); the skipjack tuna, *Katsuwonus pelamis* (Linnaeus); the little tunny, *Euthynnus alletteratus* (Rafinesque); and the frigate mackerel, *Auxis* sp. Larvae of the albacore, *Thunnus alalunga* (Bonnaterre) were not collected. Numbers of larvae, their location, and the methods used to collect, sort, identify, and compute the numbers of larvae have been treated by Richards et al. (1969a, 1969b, 1970) for each cruise. Larvae were collected by an ICITA (International Cooperative Investigations of the Tropical Atlantic) 1-m plankton net, towed at the surface.

ANALYSIS OF COLLECTION TIME

The relative apparent abundance of some fish larvae is complicated by diel variations. Oblique plankton collections that sample the entire vertical distribution of a species tend to catch fewer fish larvae during the day (Ahlstrom, 1959), presumably a result of increased net avoidance. In surface collections, such as those taken during *Geronimo* 3, 4, and 5, diel vertical migrations also could be an important factor in abundance variations.

We used the Mann-Whitney U test (Siegel, 1956) to determine the probability of equal catches of tuna larvae in day and night surface tows. A ranked test such as this should minimize the effects of patchiness. Tows with local apparent midtimes from 0600 through 1759 hr were designated as day tows and those with local apparent midtimes from 1800 through 0559 hr as night tows. Included in our calculations were all successful tows (those that captured tuna larvae) and unsuccessful tows (those that did not capture tuna larvae), except those unsuccessful tows outside the temperature-salinity ranges of the species (Table 1). These temperature-salinity ranges are a composite from Richards (1969) and the present study and should not be considered absolute. The unsuccessful tows were included because of the implication that larvae were not captured for some reason other than intolerance to temperature or salinity. In calculating the statistics, a correction for the tied (equally ranked) unsuccessful tows was used (Siegel, 1956).

TABLE 1.—Temperature-salinity ranges for larvae of yellowfin tuna, bigeye tuna, skipjack tuna, little tunny, and *Auxis* sp. These data are a composite from Richards (1969) and the present study.

Species	Temperature range	Salinity range
	°C	‰
Yellowfin tuna	23.6-29.7	33.5-36.8
Bigeye tuna	23.6-30.5	31.8-36.4
Skipjack tuna	23.4-29.7	31.4-36.9
Little tunny	22.7-29.3	32.7-35.4
Auxis sp.	21.6-30.5	33.2-35.9

The resulting probabilities (Table 2) indicate that yellowfin and bigeye tunas were collected more often at the surface during the day, and skipjack tuna and little tunny more often at the surface at night. No difference was apparent between day and night tows for *Auxis*.

Also analyzed was whether tuna larvae were better able to dodge the plankton net during the day than at night. The question was considered because we naturally assumed that tuna larvae should be able to see a plankton net more clearly during the day and therefore avoid it more

TABLE 2.—Probabilities of equal catches of larvae in day and night plankton tows.

Species	Number of day tows	Number of night tows	Total number of standardized larvae per number of successful tows		Probability of equal catches
			Day	Night	
Yellowfin tuna	263	194	1701.7/113	645.8/57	<0.01
Bigeye tuna	265	197	409.6/68	186.4/32	=0.02
Skipjack tuna	267	206	33.3/12	364.7/38	<0.01
Little tunny	274	209	303.5/28	362.2/36	=0.03
Auxis sp.	280	218	3637.8/128	2812.3/93	=0.99

easily. We also reasoned that large larvae, being better swimmers than smaller larvae, should have been captured less frequently in day collections than in night collections. Thus, if net avoidance was demonstrable, the lengths of larvae caught during the day should have been smaller.

Percent length frequencies of each species of larvae collected in the day and night were plotted from the following data: Yellowfin tuna, 1,009 day-caught larvae, 340 night-caught larvae (Figure 2); bigeye tuna, 271 day, 84 night (Figure 3); skipjack tuna, 22 day, 197 night (Figure 4); little tunny, 134 day, 72 night (Figure 5); and *Auxis*, 1,636 day, 1,082 night (Figure 6).

The length frequencies of night-caught larvae tended to be skewed more toward the larger sizes than did the day-caught larvae. The bimodal frequency of skipjack tuna captured during the day could have been due to the small sample size. The Mann-Whitney U test (Siegel, 1956) was applied to the frequencies to determine if night-caught larvae were significantly larger than day-caught larvae. Probabilities of less than 0.01 that larvae were the same length were found for yellowfin tuna, little tunny, and *Auxis*; bigeye and skipjack tunas had probabilities of 0.06 and 0.22, respectively. It should be noted also that the largest larvae of every species but bigeye tuna were captured at night. We tentatively conclude that there was greater net avoidance during the day for yellowfin tuna, little tunny, and *Auxis*, but little net avoidance for bigeye and skipjack tunas.

A differential vertical migration on the basis of size also should be considered as a possible explanation for the capture of larger larvae at night. Certain evidence causes us to reject this possibility, however. Ueyanagi (1969) found

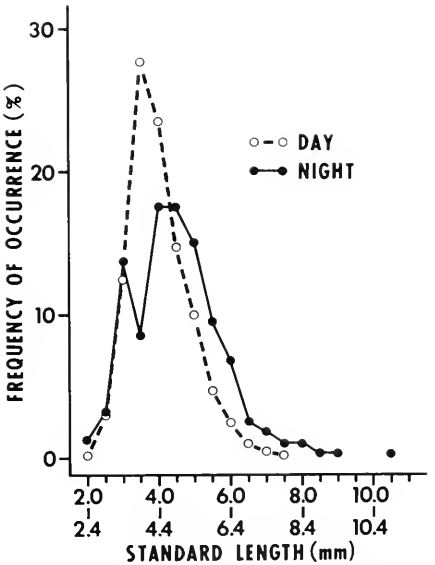


FIGURE 2.—Percent length frequencies of yellowfin tuna larvae captured during the day (broken line, 1,009 specimens) and night (solid line, 340 specimens).

that the size composition of tuna larvae taken in night surface tows resembled the size composition of those taken during both day and night at depth. Smaller larvae were more numerous in catches made at the surface during the day. The implication is that net avoidance of larger larvae is greater at the surface during the day, and there is no indication of a vertical migration of the two size groups in opposition to one another.

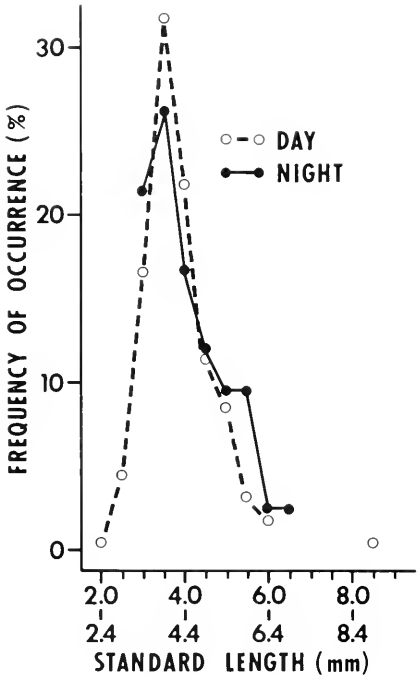


FIGURE 3.—Percent length frequencies of bigeye tuna larvae captured during the day (broken line, 271 specimens) and night (solid line, 84 specimens).

Analyses of our data show that yellowfin tuna larvae were more successfully captured in day tows than at night, even though greater net avoidance during the day was indicated. Had net avoidance been the major factor in day-night differences in abundance, more larvae should have been captured at night. Apparently —since the opposite is indicated—yellowfin tuna larvae migrate to the surface in the day and net avoidance is of minor importance, in terms of numbers collected. Ueyanagi (1964) suggested that istiophorid larvae behave similarly; other workers (Wade, 1951; Strasburg, 1960; Klawe, 1963; Ueyanagi, 1969) found no decisive evidence to show that yellowfin tuna larvae perform a vertical diel migration to the surface.

Our study indicated that bigeye tuna larvae —like those of yellowfin tuna—migrate vertically to the surface in the day, but the probabilities were not as significant ($P = 0.02$ compared with $P < 0.01$ for yellowfin tuna). Net avoidance was negligible for bigeye tuna larvae. Ueyanagi (1969) reported a greater larval ocurrence of bigeye tuna at the surface during the day than at night.

Our evidence showed that skipjack tuna larvae migrate vertically to the surface at night and that net avoidance was apparently negligible. A vertical migration to the surface at night also was suggested by Wade (1951) and Strasburg (1960). Ueyanagi (1969) reported a scarcity at the surface during the day, but increased abundance at night.

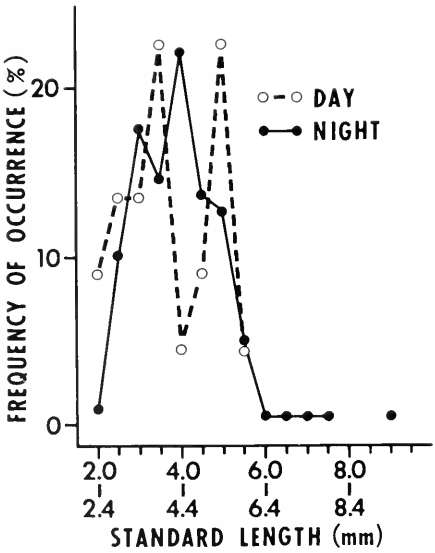
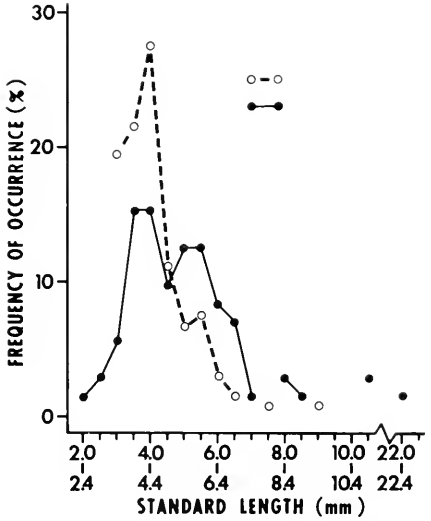


FIGURE 4.—Percent length frequencies of skipjack tuna larvae captured during the day (broken line, 22 specimens) and night (solid line, 197 specimens).



Our night tows caught little tunny larvae more successfully than day tows, but differences were not as pronounced as they were for skipjack tuna larvae ($P = 0.03$ compared with $P < 0.01$ for skipjack tuna larvae). Since a greater ability to dodge the net during the day was indicated, day-night differences could have been caused by migration to the surface at night, net avoidance, or a combination of both. Among larvae of yellowfin tuna, bigeye tuna, skipjack tuna, and *Auxis*, net avoidance was negligible or ineffective in detecting day-night differences in abundance. The higher frequency of night captures of little tunny larvae, therefore, was probably caused primarily by vertical migration to the surface at night. Vertical migration to the surface at night also was suggested for the closely related *Euthynnus yaito* (=*E. affinis*) by Wade (1951).

FIGURE 5.—Percent length frequencies little tunny larvae captured during the day (broken line, 134 specimens) and night (solid line, 72 specimens).

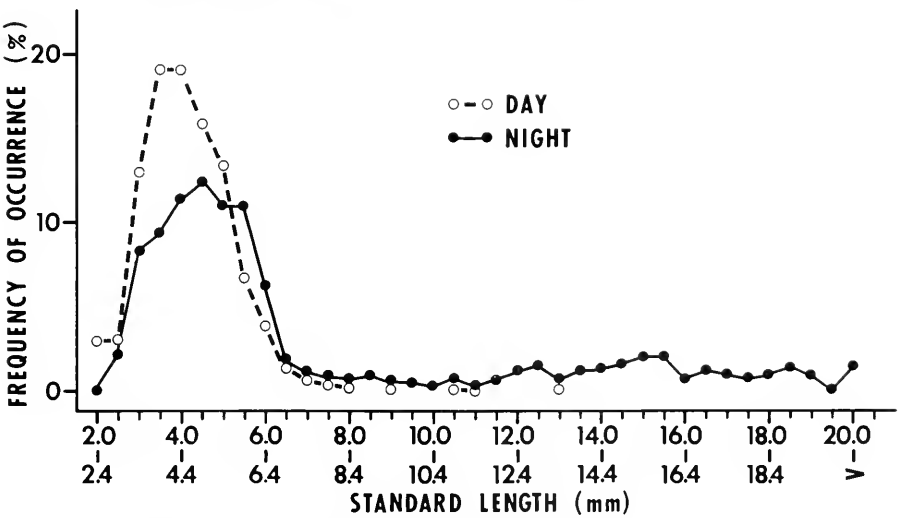


FIGURE 6.—Percent length frequencies of *Auxis* larvae captured during the day (broken line, 1,636 specimens) and night (solid line, 1,082 specimens).

Auxis larvae were equally abundant in day and night tows, indicating that this species does not migrate to the surface. The indication of net avoidance during the day had no detectable effect on apparent abundance, but if *Auxis* larvae were more abundant at the surface during the day and net avoidance had a significant effect on abundance, the same results could be obtained. Larval *Auxis* were almost equally abundant at the surface in day and night collections according to Wade (1951). Strasburg (1960) captured more *Auxis* larvae in 0 to 60 m tows at night and stated that Matsumoto (1958) also captured more specimens in night surface tows. Klawe (1963) reported greater success in catching *Auxis* larvae at night in surface and 300-m oblique tows but not in 140-m oblique tows; he suggested that net avoidance may be primarily responsible for decreased day catches. In a more recent study, Klawe, Pella, and Leet (1970) concluded that *Auxis* larvae did not exhibit a diel vertical movement; they also found no indication of net avoidance.

DISTRIBUTION OF LARVAE

Because all our collections were made by surface tows, it was not possible to directly compare our totals with the number of larvae collected during the Equalant surveys (Richards, 1967, 1969). The two multiship Equalant surveys covered most of the tropical Atlantic Ocean. Equalant I took place at the same time of year as *Geronimo* cruises 3 and 5 ("warm season"), Equalant II corresponded to the time of *Geronimo* cruise 4 ("cool season"). The average number of tuna larvae collected per 1,000 m^3 of water strained on each *Geronimo* cruise herein discussed and the average under 1 are (100 m^2) of sea surface for Equalant I and Equalant II (Richards, 1969) are shown in Table 3. The average numbers of larvae collected on the *Geronimo* cruises were corrected for diel variations in abundance. This was computed by the following formula:

$$\frac{a\,b + a'\,b'}{2}$$

where a = total number of standardized day-caught larvae
a' = total number of standardized night-caught larvae
b = total number of day tows
b' = total number of night tows.

The correction was applied to all species except *Auxis* because that species was equally abundant in day and night collections. The averages for *Auxis* were obtained by dividing the total number of standardized larvae by the total number of tows. The Equalant averages were not corrected for diel variations in abundance because most of the collections were oblique and sampled the entire vertical range of all tuna larvae. Calculations for the average number of larvae collected were similar to those used for *Auxis* but were expressed as the number under 1 are of sea surface. In the following separate accounts we report on our detailed findings concerning each species of larval tuna.

TABLE 3.—The average number of tuna larvae collected on *Geronimo* cruises 3, 4, and 5 and the two Equalant surveys.

Species	*Geronimo* cruise				Equalant survey	
	3	4	[1]5	[2]5	I	II
	Number per 1000 m^3				*Number under 1 are*	
Yellowfin tuna	11.4	5.2	1.1	1.0	7.82	5.05
Bigeye tuna	2.9	0.9	0.4	0.6	3.00	1.24
Skipjack tuna	2.3	0.3	0.1	0.2	13.71	7.85
Little tunny	3.5	0.8	0.4	0.4	--	--
Auxis sp.	12.6	9.9	6.5	18.8	--	--

[1] 14 March to 19 April 1965 in northwestern Gulf of Guinea.
[2] 10 February to 2 March 1965 off Sierra Leone.

YELLOWFIN TUNA LARVAE

The distribution of yellowfin tuna larvae in the northwestern Gulf of Guinea is shown in Figure 7. During *Geronimo* cruise 3, yellowfin tuna larvae were common throughout most of the area, averaging 11.4 larvae per 1000 m^3 of water strained. During cruise 5 (in the Gulf of Guinea a year later), a smaller area was sampled and an average of 1.1 larvae was collected per 1000 m^3 of water strained. During Equalant I, no larvae were found north of about lat 2° N in the same area, in contrast to the distribution found during *Geronimo* cruise 3. We presume

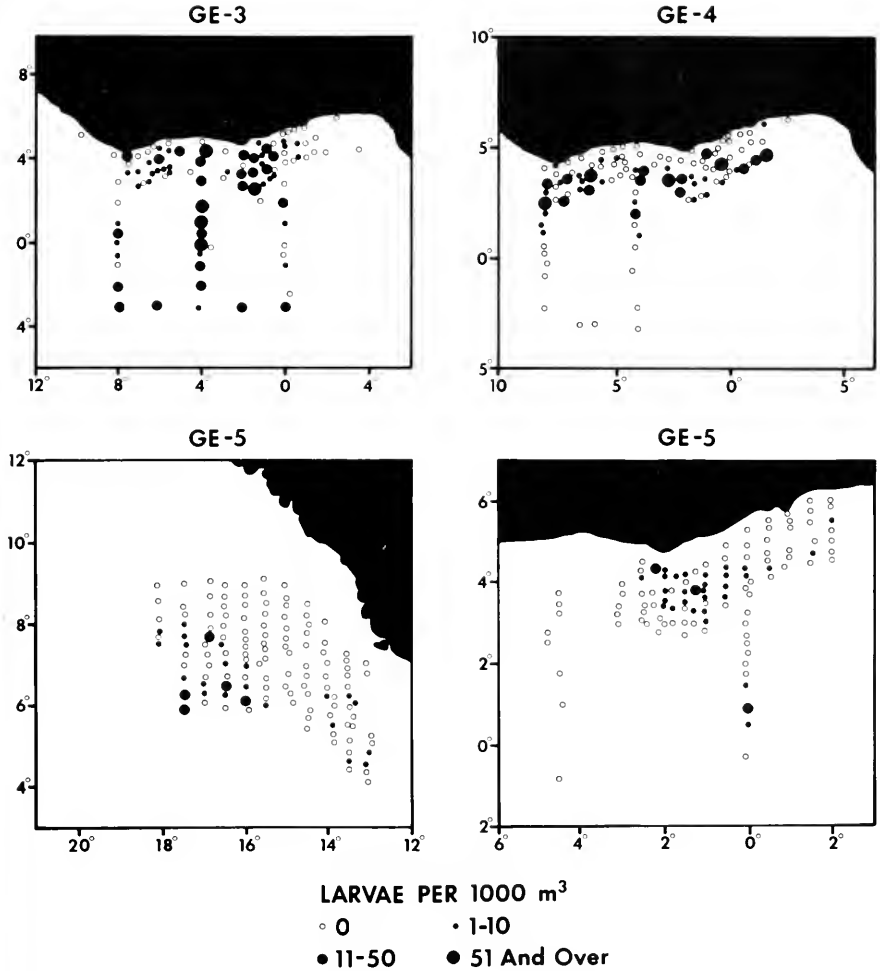


FIGURE 7.—The distribution of yellowfin tuna larvae in the northwestern Gulf of Guinea based on collections during *Geronimo* cruises 3 (10 February to 26 April 1964), 4 (5 August to 13 October 1964), 5 (14 March to 19 April 1965), and cruise 5 off Sierra Leone (10 February to 2 March 1965).

that the difference was because of increased sampling intensity on *Geronimo* cruise 3. Widespread spawning was seen near the equator, however, on both Equalant I and *Geronimo* cruise 3. An indication of this equatorial spawning is evident in cruise 5 (Figure 7). During *Geronimo* cruise 4, the distribution of larvae was reduced from that seen on cruise 3, averaging 5.2 larvae per 1000 m^3 of water strained. Again the situation differed from that found in Equalant II during which almost no larvae were taken, probably because of light sampling.

Richards (1969) found no yellowfin tuna larvae in waters with temperatures lower than 26° C, and indicated that the presence of yellowfin tuna larvae may depend on water temperature. During the *Geronimo* cruises, with one exception, yellowfin tuna larvae were collected in waters warmer than 24° C. Hence, the lower limit of 26° C for surface temperature set for the presence of yellowfin tuna larvae by Richards (1969) should be lowered to 24° C. Surface water temperatures were above 27° C at all stations sampled during cruise 3, and yellowfin tuna larvae were found between 27.9° and 29.7° C. During cruise 5 (also the "warm season"), surface temperatures ranged from 22.5° to 29.9° C but yellowfin tuna larvae were found within a range of 24.9° to 29.5° C. During cruise 4 (the "cool season"), surface temperatures ranged from 19.3° to 25.5° C; yellowfin tuna larvae were found only in water with temperatures higher than 24° C except at one station with a temperature of 23.6° C. During cruises 3, 4, and 5, surface salinity values ranged from 33‰ to 36‰. The yellowfin tuna larvae were rarely encountered when salinity fell below 34‰ but were common between 34‰ and 36‰.

In the area off Sierra Leone, yellowfin tuna larvae were encountered in water temperatures higher than 25° C (Figure 7), the area south of the 25° C isotherm. That area was not covered during the "cool season" by *Geronimo* cruises but did receive minor coverage on Equalants I and II, which resulted in the collection of some tuna larvae, particularly on Equalant II. Water temperatures were 26° C or higher at the Equalant stations where collections were made. Conand (1970) found yellowfin tuna larvae in waters warmer than 27° C off Senegal.

The Gulf of Guinea and contiguous waters account for much of the Atlantic tuna catch. Beardsley's (1969) discussion of the relation of oceanographic features to adult yellowfin tuna distributions in that area is of interest to the present study. In his summary charts of adult yellowfin tuna distributions, some catch rates are high in areas of cool water where the larvae do not occur, which indicates that an abundance of adults may not indicate abundance of larvae. Surface fishing was carried out by the *Geronimo* during cruises 3, 4, and 5 and it was interesting to note that there was no apparent relation between sightings of surface schools and location of larvae.

BIGEYE TUNA LARVAE

The distribution of bigeye tuna larvae in the northwestern Gulf of Guinea approximated that of yellowfin tuna larvae (Figure 8), but the average number per 1000 m^3 of water strained was less than for yellowfin tuna larvae. (A similar pattern was noticed on the Equalant surveys). Off Sierra Leone, the species was collected as often as yellowfin tuna (29 bigeye tuna stations compared with 28 yellowfin tuna stations), but the average number of bigeye tuna larvae collected was less than that of the yellowfin tuna. Larvae of bigeye tuna—like the yellowfin tuna larvae—were collected offshore, south of the 25° C isotherm. The apparent abundance of bigeye tuna larvae, compared with yellowfin tuna larvae, closely resembles that of the adults, as shown in the Japanese Atlantic longline data (Wise[3]). During the Equalant surveys, 3.0 times more yellowfin tuna larvae than bigeye tuna larvae were captured. In 1963 (the year of Equalants I and II) 3.4 times more yellowfin tuna adults than bigeye tuna adults were captured by Japanese longliners in the same general area (Wise, see footnote 3). During the *Geronimo* surveys 3.9 times more yellowfin tuna larvae than bigeye tuna larvae were captured. In 1964 (the

[3] Wise, J. P. 1969. Some basic statistics of the Atlantic tuna fisheries. B.C.F. Tropical Atlantic Biological Laboratory, [Miami, Fla.,] Data Summary No. 8, 14 p. [Processed.]

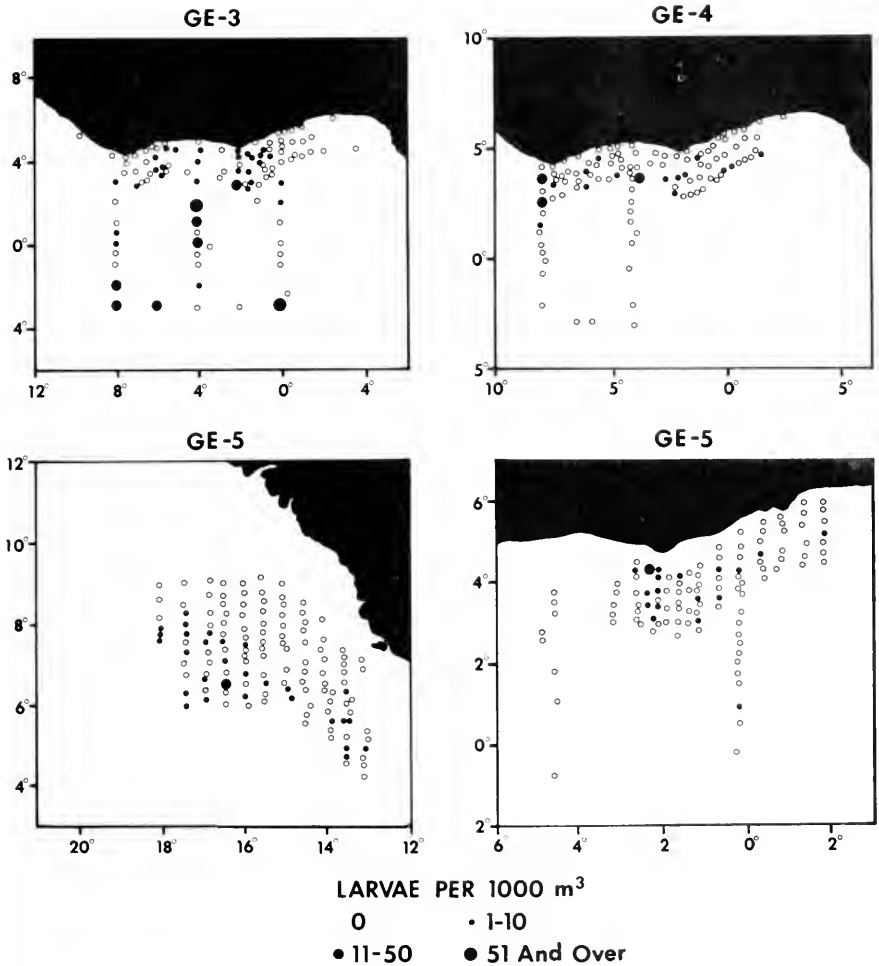


FIGURE 8.—The distribution of bigeye tuna larvae in the northwestern Gulf of Guinea based on collections during *Geronimo* cruises 3 (10 February to 26 April 1964), 4 (5 August to 13 October 1964), 5 (14 March to 19 April 1965), and cruise 5 off Sierra Leone (10 February to 2 March 1965).

predominant year of the *Geronimo* surveys), 3.7 times more adult yellowfin tuna than adult bigeye tuna were captured by Japanese longliners in the same general area (Wise, see footnote 3).

SKIPJACK TUNA LARVAE

Richards (1969) found that distributions of skipjack tuna larvae differed from those of yellowfin and bigeye tunas, particularly when surface temperature values were below 26° C. Apparently skipjack tuna larvae are able to tolerate lower temperatures than the other two tunas. In the area covered by *Geronimo* cruise 3, distributions of larval skipjack tuna (Figure 9) were similar to those of yellowfin tuna larvae, but fewer were caught. The lesser quantities may have resulted from the sampling method used; surface collections may not adequately sample the species.

On *Geronimo* cruise 3, skipjack tuna larvae were collected in water temperatures that ranged from 27.6° to 29.7° C and salinities from 34.4‰ to 35.5‰. On *Geronimo* cruise 4 (Figure 9) the species was infrequently collected, although larvae had been commonly collected in the same region on Equalant II. Skipjack tuna larvae were found in the warmer water (24.4°-25.8° C) on *Geronimo* cruise 4, which was also true of Equalant II (see Richards, 1969: 298). On *Geronimo* cruise 5 (Figure 9) larval skipjack tunas were taken at only four stations, presumably an artifact of the sampling method. Off Sierra Leone, these larvae were found at only seven stations, perhaps again an artifact of sampling.

LITTLE TUNNY LARVAE

Little tunny larvae were collected during the Equalant surveys but the data have not yet been evaluated. In the northwestern Gulf of Guinea and off Sierra Leone, little tunny larvae were collected during each *Geronimo* cruise (Figure 10). Unlike the other species, they were not collected on the outer transects near the equator. The distribution of the larvae of this species, as it related to temperature and salinity, was also noticeably different from that of the other tuna species. The temperatures for the larvae ranged from 22.7° to 29.3° C and the salinities from 32.7‰ to 35.4‰. Apparently little tunny larvae can tolerate a wider range of physical conditions than can the larvae of the more oceanic tunas—skipjack, yellowfin, and bigeye.

Auxis sp.

Larvae of *Auxis* (frigate mackerels) are unquestionably the most abundant scombrid larvae found in these tropical waters. This abundance holds true for the eastern Pacific (Klawe, 1963), as well as for the eastern Atlantic (Figure 11). (We are aware that *Auxis* may be two species, but as yet methods for distinguishing their larvae have not been satisfactorily developed.) In the northwestern Gulf of Guinea, *Auxis* larvae were collected mostly nearshore, though a few specimens were found offshore. *Auxis* was the only species widely distributed off Sierra Leone. One reason for its abundance may be the wide tolerance of the larvae for temperature and salinity—*Auxis* larvae were found in water with temperatures as low as 24.6° C and as high as 30.5° C, the widest temperature range found for any tuna larvae we studied. The salinity range of the species was 33.2‰ to 35.9‰.

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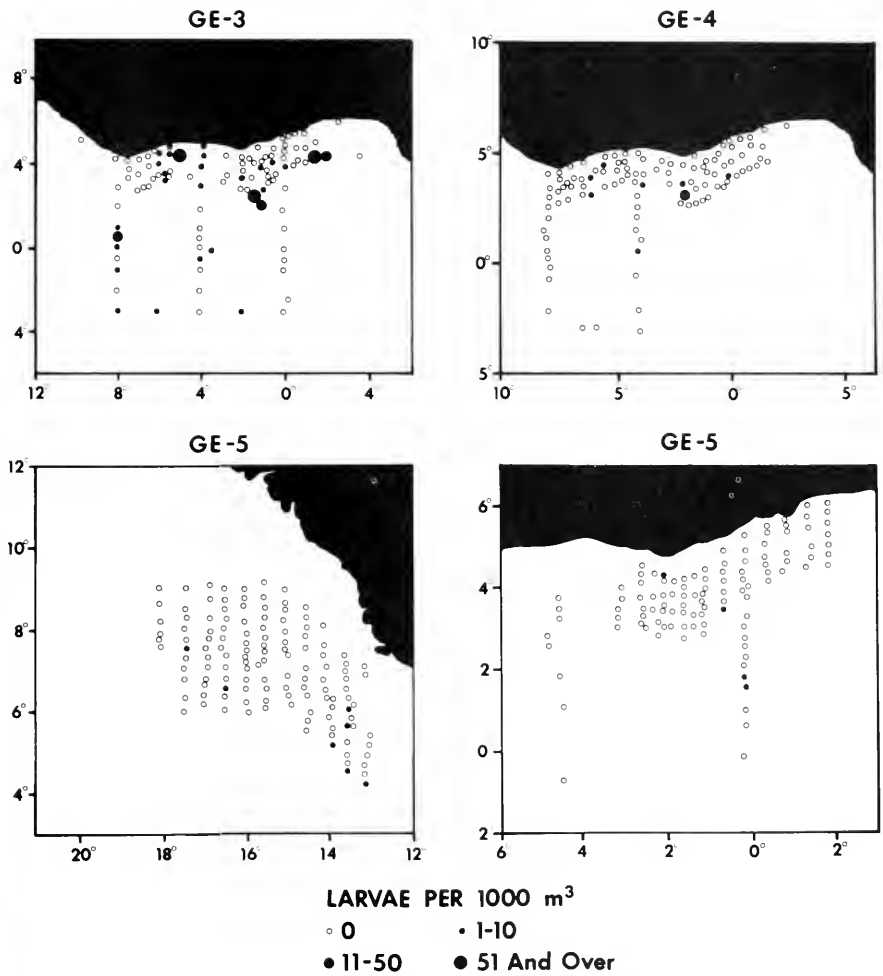

FIGURE 9.—The distribution of skipjack tuna larvae in the northwestern Gulf of Guinea based on collections during *Geronimo* cruises 3 (10 February to 26 April 1964), 4 (5 August to 13 October 1964), 5 (14 March to 19 April 1965), and cruise 5 off Sierra Leone (10 February to 2 March 1965).

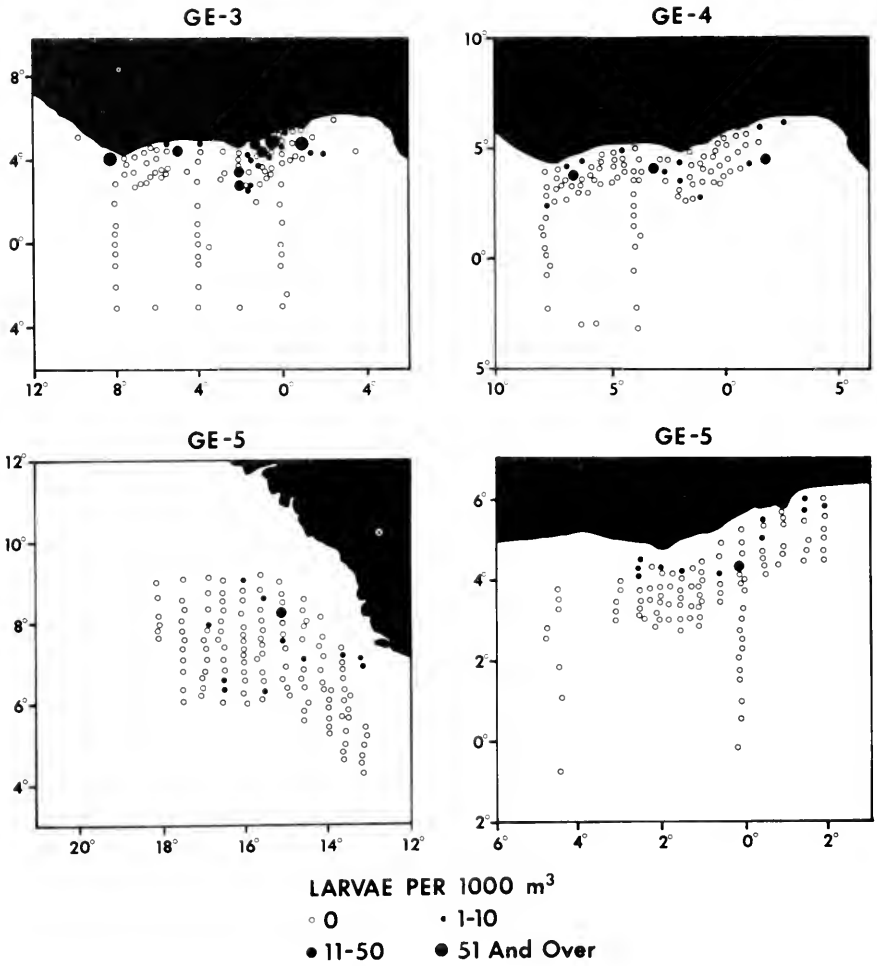

FIGURE 10.—The distribution of little tunny larvae in the northwestern Gulf of Guinea based on collections during *Geronimo* cruises 3 (10 February to 26 April 1964), 4 (5 August to 13 October 1964), 5 (14 March to 19 April 1965), and cruise 5 off Sierra Leone (10 February to 2 March 1965).

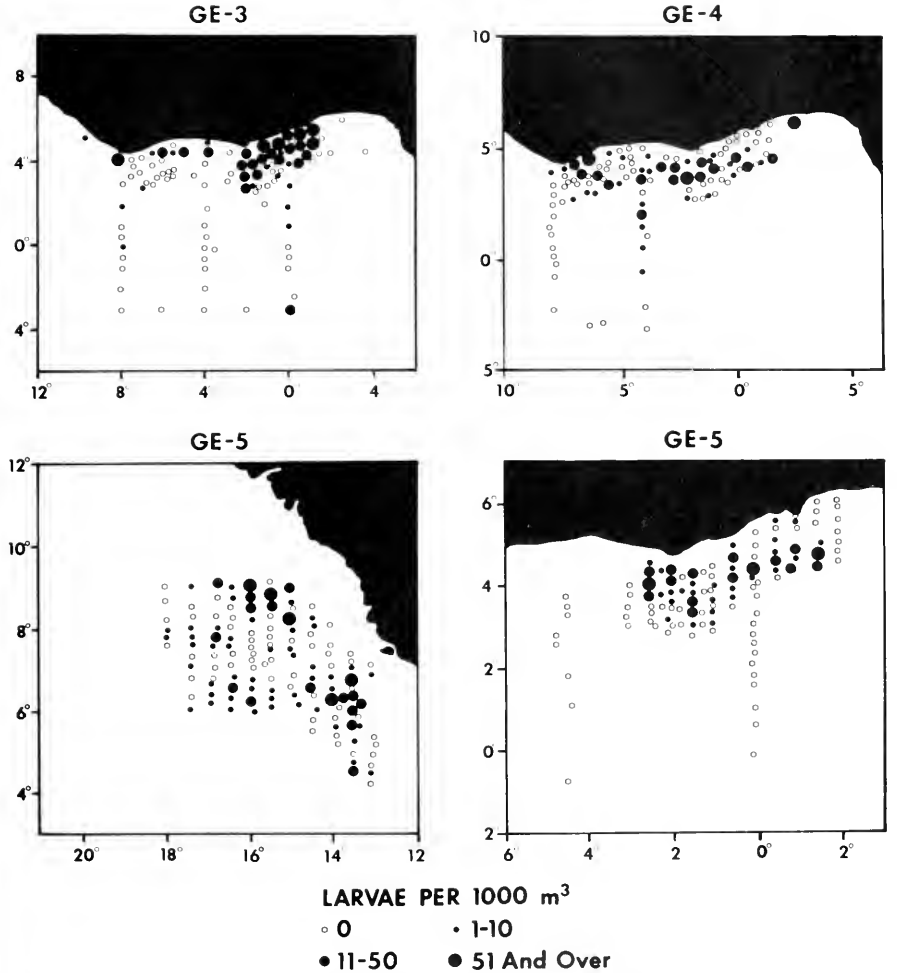

FIGURE 11.—The distribution of frigate mackerel larvae, *Auxis* sp., in the northwestern Gulf of Guinea based on collections during *Geronimo* cruises 3 (10 February to 26 April 1964), 4 (5 August to 13 October 1964), 5 (14 March to 19 April 1965), and cruise 5 off Sierra Leone (10 February to 2 March 1965).

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RANDOM VARIABILITY AND PARAMETER ESTIMATION FOR THE GENERALIZED PRODUCTION MODEL[1]

WILLIAM W. FOX, JR.[2]

ABSTRACT

Three alternative statistical models are proposed for estimating the parameters of the generalized production model by the method of least squares. A stochastic representation of the generalized production model is constructed and simulation (or the Monte Carlo Method) is employed to infer the effects of random variability on the variation in catch. The use of residuals examination for selecting the appropriate statistical model for least-squares estimation of the generalized production model parameters is demonstrated for the yellowfin tuna fishery in the eastern tropical Pacific Ocean. In both the simulation and actual fishery, statistical Model 3—assuming catch residual variance is proportional to the catch squared—best fulfills the assumptions of least-squares theory and should, therefore, provide the best least-square parameter estimates.

Mathematical models are powerful tools which are being used increasingly in resource management. A knowledge of mathematics allows a resource manager to construct from gathered data a representation of the real system and, coupled with statistical theory, allows estimation of the parameters of his model. Then, as is impossible in the real system, a manager may experiment on his model and derive outcomes which aid decisions about management of the real system. Results of model experimentation usually depend greatly on the formulation of the model and to some degree on the accuracy of the parameter estimates. Often precise statistical parameter estimation lags behind mathematical formulation, primarily because many mathematical models are robust, i.e., decisions are independent of parameter accuracy. This is one reason for the development of deterministic rather than stochastic models. However, it seems that it is always desirable to obtain the best possible parameter estimates from the data at hand.

A simple case of Bernoulli's equation has been suggested as a model for the growth of an organism by Richards (1959), Chapman (1961), and Taylor (1962)

$$dx/dt = Hx_t^m - Kx_t \qquad (1)$$

where x_t represents either weight or length at time t, and H, K, and m are parameters which may be given some physiological significance. Recently equation (1) has been advanced independently by Chapman (1967) and Pella and Tomlinson (1969) as a simple model for assessing the relation between exploitation and yield (or catch) from a living resource

$$dP/dt = HP_t^m - KP_t - qfP_t \quad \text{for } m < 1$$
$$dP/dt = -HP_t^m + KP_t - qfP_t \quad \text{for } m > 1 \qquad (2)$$

where P_t is the population size (biomass or numbers), f is the amount of fishing effort, q is the coefficient of catchability, and H, K, and m are parameters. It is assumed that f is constant over the time period that equation (2) is used. Therefore, $qf = F$, the instantaneous fishing mortality coefficient, and $qfP_t = C_t$ the catch. Equation (2), referred to herein as the generalized production model after Pella and Tomlinson (1969), includes the logistic model used by

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Graham (1935), Schaefer (1954, 1957), and others when $m = 2$, and the exponential model discussed by Fox (1970) if the limit is taken as $m \rightarrow 1$.

This type of production modeling is a stock assessment approach which has extreme mathematical and data requirement simplicity. Therein lies its primary virtue; for example, equation (2) contains only four parameters whereas the simplest Beverton and Holt (1957) type of model providing the same relation contains at least nine parameters. Estimation of the parameters of equation (2) requires only catch and fishing effort data while at the very least, the Beverton and Holt approach additionally requires age structure information. Discussion of the different assumptions for implementing each approach can be found in Schaefer and Beverton (1963). The generalized production model provides for a wide variety of shapes for the production curve and thus coupled with its mathematical simplicity represents an important tool for successfully managing exploitation.

Procedures for estimating the parameters of production models can be found in Schaefer (1954, 1957), Ricker (1958), Chapman, Myhre and Southward (1962), Gulland (1969), and Pella and Tomlinson (1969). However, it appears that in all cases, except Schaefer (1957), random variation about the deterministic predictions of the production model has been largely ignored in choosing a *statistical* model for estimating the parameters. Perhaps this is because of the apparent formidable nature of such variation. On the other hand, such variation may often be approximated in a simple manner to allow better estimates of the parameters than if ignored altogether. It is conceded that the generalized production model is at the very best only a good approximation of the actual biological dynamics, but this should not imply that better parameter estimates are unwarranted, unless its prime virtue of mathematical simplicity is compromised in the course of such action.

Several statistical models for estimating the parameters of mathematical models of biological relationships have been discussed variously by Zar (1968), Glass (1969), Hafley (1969), and Pienaar and Thomson (1969). While to the nonstatistician these papers may bear a strong resemblance to quibbling over apparent minor differences of results in the face of large data variability, the improper statistical model can lead to misleading conclusions or to significant errors, as several of the above authors demonstrated. Statistical models differ on the assumption about the manner in which variation or error enters the deterministic biological model. The technique employed by Pienaar and Thomson (1969) to assess fulfillment of the assumptions about variation is the graphing and examination of residuals, the differences between the observed data and those predicted by the model. Extensive discussion on the examination and analysis of residuals can be found in Anscombe (1961), Anscombe and Tukey (1963), and Draper and Smith (1966).

This paper presents a discussion of the nature of simple random variability and its relation to estimating the parameters of the generalized production model. An illustration of residuals examination in selecting the appropriate statistical model for the parameter estimating technique of Pella and Tomlinson (1969) is included. Data from the fishery for yellowfin tuna, *Thunnus albacares*, in the eastern tropical Pacific Ocean were utilized in the illustration.

STATISTICAL MODELS

Schaefer (1957) recognized that the production model is not deterministic and represented environmentally induced variation as an additive term consisting of a random variable η multiplied by population size. In terms of the generalized production model

$$dP/dt = KP_t - HP_t^m - qfP_t + \eta P_t \,. \quad (3)$$

His parameter-estimating procedure used a finite difference approximation of equation (3) divided through by P_t for the case when $m = 2$. By summing over many time periods the effects of variation are eliminated since the expected value (or mean) of η is zero. Schaefer's formulation of the error term, while reasonable and convenient for his estimating technique, pro-

duces a complex statistical model on integrating equation (3). Therefore, his statistical model was given no further consideration.

Pella and Tomlinson (1969) also mentioned that the generalized production model is not deterministic. They pointed out several sources of error in Schaefer's finite difference approximation of population change and estimation procedure, and advanced a "least-squares" searching procedure as an alternative. In doing so, however, apparently no consideration was given to statistical implications of their technique. The Pella-Tomlinson procedure integrates equation (2) over the time period during which the fishing effort is assumed constant, Δt, to give

$$\hat{P}_t = \left[\frac{H}{K(\pm)\,qf} - \left(\frac{H}{K(\pm)\,qf} - P_0^{1-m}\right) \times e^{\mp(K(\pm)\,qf)(1-m)\,t}\right]^{\frac{1}{1-m}} \quad (4)$$

where P_0 is the population size at the beginning of the time period, and the upper signs applying when $m < 1$ and the lower when $m > 1$. Starting with initial guesses of the parameter values, an estimated catch history, $\{\hat{C}_i\}$ where $i = 1 \ldots n$ time periods, is calculated from the known fishing effort history, $\{f_i\}$, by the formula

$$\hat{C}_i = qf_i \cdot \sum_{j=1}^{N} \frac{1}{2}(\hat{P}_{i,j} + \hat{P}_{i,j+1}) \cdot \Delta t_i / N \quad (5)$$

where $\hat{P}_{i,j}$ are found from equation (4) over $j = 1 \ldots N$ subintervals of each time interval i. The fitting criterion, S, is computed from the known catch history, $\{C_i\}$, of n time periods as

$$S = \sum_{i=1}^{n} (C_i - \hat{C}_i)^2 = \sum_{i=1}^{n} \epsilon_i^2 \quad (6)$$

where the ϵ_i are residuals. The initial parameter guesses are then modified in a searching routine with their computer program GENPROD until those parameter values which minimize S are located.

The statistic S is a "least-squares" criterion. For the parameters of a nonlinear model which minimize S to be the *best* least-squares estimates, the residuals, ϵ_i, must: 1) be independent, 2) have an expected value (or mean) of zero, and 3) have constant variance (i.e., not correlated with t, $\hat{C}_i$, or f_i).[3] Consequently, the proper statistical model for the Pella-Tomlinson fitting technique must both fulfill the three assumptions and be biologically rational. It is also important that the statistical model be simple, i.e., one which requires no additional parameters to be estimated.

Ignoring for the moment that equation (5) is an approximation, the choice of equation (6) as the least-squares estimate criterion tacitly assumes

$$C_i = \hat{C}_i + \epsilon_i \quad (7)$$

giving

$$\bar{P}_i = \hat{\bar{P}}_i + (1/qf_i) \cdot \epsilon_i \quad (8)$$

where $\bar{P} = \int_0^1 P\,dt$ for ease of notation. Equation (7), referred to hereafter as statistical Model 0, is biologically tantamount to assuming random variation in population size approaches being infinitely great in an unexploited population. This denies the concept of an environmentally limited maximum population size or "carrying capacity" which is usually a foundation of the production model. Therefore, Model 0 assumed by Pella and Tomlinson is intrinsically unattractive even though it may be a reasonable approximation at intermediate exploited population levels.

There are three simple statistical models (among many) which are commonly assumed, biologically reasonable, and involve calculating S as a weighted sum of squares or from transformed data.

Model 1. Additive Error

$$\bar{P}_i = \hat{\bar{P}}_i + \epsilon_{1i} \quad (9)$$

so

$$C_i = \hat{C}_i + (qf_i) \cdot \epsilon_{1i} \quad (10)$$

[3] Additionally, if the ϵ_i are normally distributed then it can be shown that the least-squares estimates are also the maximum likelihood estimates which have minimum variance as the number of data grows large—hence are global best estimates (e.g., see Draper and Smith, 1966).

giving

$$S_1 = \sum_{i=1}^{n} [(C_i - \hat{C}_i)\ f_i]^2 \quad (11)$$

as the appropriate criterion to be minimized.

Model 2. Multiplicative Error

$$\bar{P}_i = \hat{P}_i \cdot \epsilon_{2i} \quad (12)$$

so

$$C_i = \hat{C}_i \cdot \epsilon_{2i} \quad (13)$$

or

$$\ln C_i = \ln \hat{C}_i + \ln \epsilon_{2i} \quad (14)$$

giving

$$S_2 = \sum_{i=1}^{n} (\ln C_i - \ln \hat{C}_i)^2 \quad (15)$$

as the appropriate criterion to be minimized.

Model 3. Additive Proportional Error

$$\bar{P}_i = \hat{P}_i + \hat{P}_i \cdot \epsilon_{3i} \quad (16)$$

so

$$C_i = \hat{C}_i + \hat{C}_i \cdot \epsilon_{3i} \quad (17)$$

giving

$$S_3 = \sum_{i=1}^{n} [(C_i - \hat{C}_i)\ \hat{C}_i]^2 \quad (18)$$

as the appropriate criterion to be minimized.

Model 1 assumes constant variation at all population levels. This is perhaps the least biologically reasonable of the three suggested alternative statistical models since it is easier to conceive that under equilibrium conditions a population will fluctuate more radically near its environmentally limited maximum size than at smaller sizes under constant exploitation. Model 1 is usually employed as a statistical model when variation is expected to arise from experimental or measurement error. Assuming adequate statistics of catch and fishing effort exist, it is more likely that variation will arise from environmental influences on the parameters of the model. Models 2 and 3 assume that variation in population size decreases with population size and that variation in catch increases with the size of the catch. Models 2 and 3 approximate the stochastic representation of equation (2) suggested by Pella and Tomlinson [their equation (14)]

$$dP_t/dt = \eta_1 [(\pm) HP_t^m \ (\mp)\ KP_t] - \eta_2 qfP_t \quad (19)$$

where η_1 and η_2 are continuous random variables.

Other statistical models obviously could be constructed, such as

$$\bar{P}_i = \hat{P}_i + \hat{P}_i^c \cdot \epsilon_i \quad (20)$$

where c could assume any value—Models 1 and 3 are actually special cases with $c = 0$ or 1 respectively. However, this would introduce another parameter to be estimated. The four previously described statistical models will suffice.

Returning to the point that equation (5) is a numerical approximation of integration, equations (7), (10), (13), (14), and (17) are not strictly true for the Pella-Tomlinson procedure. Accurate representations would include an additional error term due to linear approximation. However, as provided for, the linear approximation error may be reduced by increasing the value of N in equation (5). As will be demonstrated later, this error is very small in relation to the magnitude of the ϵ_i even at small values of N. The choice of N, on the other hand, can be critical to obtaining good estimates of several parameters.

We now have three alternative statistical models which fulfill the goals of simplicity and biological rationality to various degrees. It remains to be determined which of them fulfills the assumptions of least-squares theory for obtaining the best parameter estimates.

STOCHASTIC SIMULATION

An analytical solution for the appropriate statistical model is not possible since the actual causes of variability and the relationships to their effects on the generalized production model

are unknown. However, a commonly used approach, simulation (or the Monte Carlo method), may be employed to infer probable effects of variability and lead to selection of the "best" statistical model. This simulation study consisted of constructing a stochastic (or probabilistic) analogue of the generalized production model and then simulating the catches at various levels of constant fishing effort. Inferences will be drawn about the propriety of all four statistical models from residual variation produced in the catches. Also, the sensitivity of catch residual variation to parameter variation will be demonstrated.

The generalized production model can be written in a form that is more easily discussed biologically

$$dP/dt = P_t K[(P_\infty{}^{m-1} - P_t{}^{m-1})/P_\infty{}^{m-1}] - qfP_t \qquad (21)$$

The signs (+ or —) are set for convenience assuming $m > 1$. The usual biological interpretation of the constants is as follows: K is "the intrinsic rate of natural increase", $P_\infty = (K/H)^{1/(m-1)}$ is the asymptotic environmentally limited maximum population size or "carrying capacity", and m is the determinant of the proportion of P_∞ at which the maximum rate of production occurs. The stochastic analogue of equation (21) is

$$dP/dt = P_t\kappa[(\pi^{\mu-1} - P_t{}^{\mu-1})/\pi^{\mu-1}] - \gamma fP_t \qquad (22)$$

where $\{\kappa, \pi, \mu, \gamma\}$ are stochastic variables with expected values (or means) $\{K, P_\infty, m, q\}$ respectively, and distributions and variances to be specified. The parameters of equation (21) were considered to be stochastic variables since they are actually average conditions determined by many environmental inter-relationships.

The distributions and variances of the stochastic variables are unknown as are their expected values to be estimated from the fishery data. Some broad inferences about the distributions can be made, however, from biological and mathematical implications of the production model. The "intrinsic rate of natural increase", κ, was assumed to be approximately normally distributed $[\sim N(K,\sigma^2{}_1)]$, because K is the resultant rate of a linear combination of rates—birth rate — death rate (P in numbers), or birth rate + growth rate — death rate (P in biomass)—so may be either positive or negative at any given time. Negative values for π and γ are biologically and physically meaningless so they were assumed to be approximately log-normally distributed $[\sim\log N(P_\infty, \sigma^2{}_2)$ and $\sim\log N(q,\sigma^2{}_3)$ respectively]. The integrated forms of equations (2), (21), or (22) do not exist for $m = 1$; therefore μ was assumed to be given by $[1 + (m - 1)\xi]$ where ξ was assumed to be approximately log-normally distributed with a mean of one $[\sim\log N(1, \sigma^2{}_4)]$. This resulted in μ having a mean of m with a range of minus infinity to one, or one to plus infinity, depending on whether m is less or greater than one.

Integrating equation (22) from P_0 to P_t yields

$$\widehat{P}_t = \left\{ \pi^{1-\mu}\left(\frac{\kappa}{\kappa-\gamma f}\right) - \left[\pi^{1-\mu}\left(\frac{\kappa}{\kappa-\gamma f}\right) - P_0{}^{1-\mu}\right] e^{(\kappa-\gamma f)(1-\mu)t}\right\}^{\frac{1}{1-\mu}}; \qquad (23)$$

this is the stochastic analogue of equation (4). Expected values and arbitrary variances ($\sigma^2{}_1$, $\sigma^2{}_2$, $\sigma^2{}_3$, $\sigma^2{}_4$) were chosen to allow:

Stochastic variable	Expected value	Approximate 99% range
κ	5.60	5.30-5.90
μ	2.00	1.95-2.06
π (10^8)	1.40	1.07-1.83
γ (10^{-5})	7.00	6.63-7.39

The expected values were rounded approximate values obtained in the example following this section. In the same manner as the previously described Pella-Tomlinson technique, equations (5) and (23) were used to simulate a 48-year catch history at each of 13 levels of fishing effort. The continuous stochastic variable case was approximated by setting $N = 10$ in equation (5). At each iteration, the stochastic variables $\{\kappa, \pi, \mu, \gamma\}$ were drawn at random from their respective probability distributions, produced

with a random number generator by the multiplicative congruential method (subroutine RAND, University of Washington Computer Center). The variances and means of residuals and log-residuals were calculated at each fishing effort level.

The results of the simulation trials are given in Table 1. It was obvious from the formulation of equation (23) that Model 0—assuming constant residual variance—was inappropriate, the simulation trials add confirmation. Model 1—assuming residual standard deviation proportional to fishing effort—is also rejected over any moderate range of fishing effort. A close approximation, however, is obtained for $f \leqslant 22{,}000$.

Model 2—assuming constant log-residual variance—appears to be valid up to $58{,}000 \leqslant f < 65{,}000$, where a trend of increasing variance begins. The hypothesis of common log-residual variance for $f \leqslant 65{,}000$ was tested by Bartlett's t-test (Snedecor and Cochran, 1967). The result is not significant (uncorrected $\chi^2 = 7.72$, 9 df, $Pr > 0.50$). Including the log-residual variance for $f = 70{,}000$, however, significance is approached (corrected $\chi^2 = 16.35$, 10 df, $Pr < 0.10$).

Model 3—assuming residual standard deviation proportional to catch—fulfills the assumption about as well as Model 2. The proportional relationship between the residuals standard deviations and deterministic catch (Figure 1) appears to be different between catches given by fishing effort below and above that which produces the maximum sustainable yield (MSY) ($C = 196 \times 10^6$). Regression analysis reveals that variance about regression, S_{yx}^2, is highly significantly different between below and above MSY levels ($F = 9.90$; 4, 4 df; $Pr < 0.01$), but the regression coefficients, $\hat{b}$, are not significantly different ($t = 1.47$; 5 df; $Pr > 0.20$)—Table 2. The "above MSY" regression has a y-intercept, which must be zero, significantly different from zero ($t = 3.30$; 4 df; $Pr < 0.05$). It appears that Model 3, like Model 2, is valid up to $58{,}000 \leqslant f < 65{,}000$ (Figure 1).

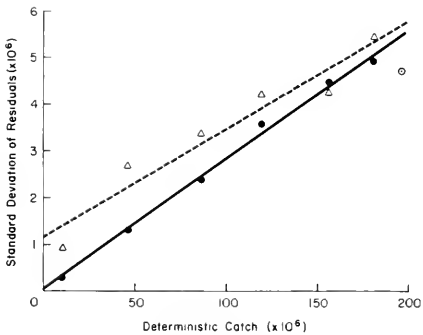

FIGURE 1.—Standard deviation of the residuals, ϵ_3, plotted against the deterministic catch, $\widehat{C}$, for statistical Model 3. • = fishing effort below maximum sustainable yield (MSY) level. △ = fishing effort above MSY level. ⊙ = fishing effort at MSY level.

TABLE 1.—Results of the stochastic catch simulation trials of the generalized production model.

Fishing effort	Deterministic catch	Deterministic population size	Mean residual		Residual variance	
	(10^6)	(10^6)	$\bar{\epsilon}$ (10^6)	$\overline{\ln \epsilon}$ (10^{-2})	$S^2(\epsilon)$ (10^{12})	$S^2(\ln \epsilon)$ (10^{-4})
f	$\widehat{C}$	$\widehat{P}$				
1,000	9.6775	138.25	−0.0706	−0.7765	0.0844	9.1231
5,000	45.9375	131.25	−0.0599	−0.1713	1.7538	8.3187
10,000	85.7500	122.50	0.0143	−0.0218	5.7506	7.8882
15,000	119.4375	113.75	0.2155	0.1357	13.0288	9.0945
22,000	156.3100	101.50	−0.7088	−0.4956	20.4779	8.3884
29,000	181.1775	89.25	−1.1161	−0.6551	24.6701	7.5760
40,000	196.0000	70.00	−0.6563	−0.3646	22.6228	5.9845
51,000	181.1775	50.75	0.5173	0.2404	30.1457	9.1269
58,000	156.3100	38.50	−0.1051	−0.1044	18.4686	7.6021
65,000	119.4375	26.25	0.5438	0.3927	18.1894	12.5489
70,000	85.7500	17.50	0.2865	0.2577	11.5283	15.4564
75,000	45.9375	8.75	−0.3734	−0.9846	7.2114	34.2285
79,000	9.6775	1.75	−0.2770	−3.4017	0.8834	102.6439

TABLE 2.—Regression analysis for statistical Model 3 of standard deviation of catch residuals, $S(\epsilon_3)$, on deterministic catch, $\hat{C}$, with levels of fishing effort below and above that which produces the maximum sustainable yield (MSY).

Effort level regression	Degrees of freedom	Σx^2	Σxy	Σy^2	$\hat{b}$	Degrees of freedom	Sum of squares	$S^2_{y/x}$
Below MSY	5	21421.9	601.047	16.9475	0.028058	4	0.083566	0.020892
Above MSY	5	21421.9	498.146	12.4109	0.023254	4	0.826986	0.206746

In conclusion, the assumption of statistical Models 0 and 1 were rejected by the simulation study. Statistical Models 2 and 3 were found to be valid over a wide and similar range of fishing effort. Their range of validity includes up to and well beyond the level of fishing effort producing the MSY ($f = 40{,}000$), the most likely range in which a fishery would operate. Employing Model 3 has a theoretical advantage over Model 2 in a least-squares estimating procedure. With Model 3, the actual residual variance is minimized. Whereas with Model 2 the log-residual variance is minimized and the parameters are best least-squares estimates only in the transformed model. The theoretical advantage of Model 3 may serve as a criterion for choosing it when no other criteria exist.

Several additional simulation trials were made to demonstrate the relative degree of influence that random variability in each parameter exerts on the variance of the catch residuals. The upper two standard deviations of each stochastic variable was set equal to 25% of their mean, the level of fishing effort was set at 40,000 (MSY-producing level), and four trials of 500 time periods each were made. Each parameter in turn was allowed to vary with the remaining three constant (Table 3). The variation in catch was most sensitive to varying the exponent, m, and least sensitive to varying the catchability coefficient, q. This, of course, implies the relative precision of the parameters if they had been actual estimates. One should not, however, generalize on the order of precision since these results obtain specifically for the assumed probability distributions and expected values. This exercise does demonstrate a frequently employed method for implying which parameters, given their estimates, are most critical and perhaps deserving of additional independent estimation.

TABLE 3.—Catch residual variance produced by variation in each stochastic variable of the generalized production model.

Stochastic variable	Expected value	Approximate 95% range	Residual variance	
			$S^2(\epsilon)$ (10^{12})	$S^2(\ln \epsilon)$ (10^{-4})
μ	2.00	1.67-2.50	46.0834	11.9100
κ	5.60	4.20-7.00	36.8604	9.7958
π (10^8)	1.40	1.12-1.75	30.7556	8.1498
γ (10^{-3})	7.00	5.60-8.75	17.1333	4.4781

RESIDUALS EXAMINATION: AN EXAMPLE

The data of catch, catch per unit effort, and fishing effort from the eastern tropical Pacific yellowfin tuna fishery (Pella and Tomlinson, 1969; Table 6) are plotted in Figure 2. Apparently the population and fishery dynamics are

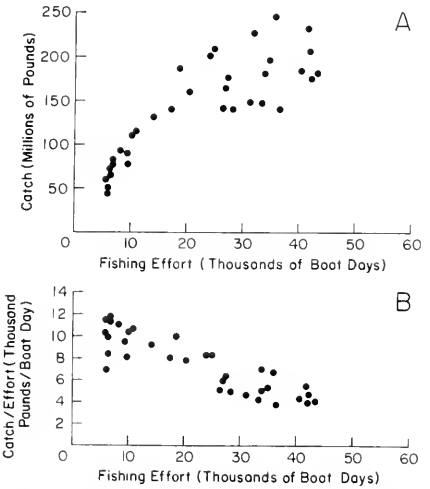

FIGURE 2.—Data from the eastern tropical Pacific yellowfin tuna fishery, 1934-67, plotted as (A) catch vs. fishing effort, and (B) catch per unit effort vs. fishing effort.

well described by a production model—good relationships are observed in Figure 2. These data were used by Pella and Tomlinson in exemplifying their technique; for comparative purposes the same data are utilized here. The results of this section, however, should be considered as just an example and not a recommendation on management.

The parameters of the generalized production model for the tuna fishery were estimated by the Pella-Tomlinson computer program, GENPROD, replacing the fitting criterion, S, with those of each alternative statistical model [equations (11), (15), and (18)]. Each parameter was searched to five digits or until the improvement in S was less than 0.01% at three levels of numerical approximation in equation (5)—$N = 1, 3, 5$—(Table 4). Increasing the precision of numerical approximation greatly changed the parameter estimates between $N = 1$ and 3, but only slightly between $N = 3$ and 5. The most sensitive parameter is H, followed in order by K, q, m, and r. Consequently, the estimates of the average environmentally limited maximum population size, $\bar{P}_\infty$, and average optimum population size $\bar{P}_{opt}$, vary with the level of precision. Pella and Tomlinson indicated that unreasonable estimates were obtained for the catchability coefficient, q, (presumably with $N = 1$) and made an arbitrary selection of a "reasonable" estimate. "Reasonable" catchability coefficients are obtained here with $N = 3$, making unnecessary the arbitrary selection of a reasonable estimate. The management implications of maximum equilibrium catch, C_{max}, and optimum fishing effort, f_{opt}, are surprisingly robust to the degree of precision of the numerical approximation. Schaefer (1957) mentioned previously, however, that these two management implications are robust to changes in the estimate of q in his estimating method; Pella and Tomlinson also mentioned the phenomenon for their technique. The S criteria values were reduced about 7% or less by choosing $N = 3$, as against $N = 1$ and reduced a negligible 0.2% or less by choosing $N = 5$ (S_1 and S_2 increased minutely due to the level of precision chosen for S). Obviously, the error due to approximation in equation (5), as previously stated, is negligible for these data with $N \geq 3$.

Turning to the effects of the alternative statistical models (with $N = 5$), it may be seen

TABLE 4.—Parameters and management implications of the generalized production model for the eastern tropical Pacific yellowfin tuna fishery, 1934-67, estimated with the Pella-Tomlinson technique (GENPROD) using four different statistical models and three levels of precision, N, in equation (5).

Model	Parameters					Management implications				S Criterion
	$\hat{m}$	$\hat{H}$ (10^{-5})	$\hat{K}$	^1r	$\hat{q}$ (10^{-5})	$\bar{P}_\infty$ Pounds (10^6)	C_{max} Pounds (10^6)	f_{opt} Boat days	$\bar{P}_{opt}$ Pounds (10^6)	
					$N = 1$					
20	1.4	--	--	--	45.--	--	182.6	35,300	--	1.78--$\times 10^{16}$
0	1.4	2900.1	33 26	.820	27.00	44 6	182.6	35,200	19.2	1.7858$\times 10^{16}$
1	1.9	0.17064	15.21	.904	21.69	52.7	186.2	33,210	25.8	4.7140$\times 10^{7}$
2	1.6	34 502	16 65	.879	18.10	64.0	182.5	34,500	29.2	8.2214$\times 10^{-1}$
3	2.0	0.00748	7.57	.865	11.50	101 2	191.5	32,900	50.6	7.8762$\times 10^{-1}$
					$N = 3$					
0	1.5	59.999	7.65	.842	7.36	162.7	184 5	34,660	72.3	1.7197$\times 10^{16}$
1	2 2	0.00013	5 90	.921	10 00	113.0	188.6	32,200	58.6	4 5291$\times 10^{7}$
2	1.8	0 17092	5 86	.952	7.70	147.4	184 0	33,800	70.7	8.0355$\times 10^{-1}$
3	2.0	0.00408	5.59	.835	8.50	136.9	191.5	32,900	68.5	7.3769$\times 10^{-1}$
					$N = 5$					
0	1.5	55 802	7.28	.843	7.10	170.4	183.9	34,200	75.7	1.7185$\times 10^{16}$
1	2.2	0.00010	5.38	.921	9.11	124.0	188.7	32,210	64.3	4.5296$\times 10^{7}$
2	1.8	0.15820	5 61	.926	7.40	153.9	184.1	33,700	73.8	8.0371$\times 10^{-1}$
3	2.1	0.00054	5 06	.845	8.10	142.7	192.6	32,700	72.7	7.3608$\times 10^{-1}$

[1] $r = P_0/P_\infty$

[2] Pella and Tomlinson (1969, Table 5).

that the most sensitive estimate is H, followed in succession by the estimates of m, K, q, and r. The estimates of the management implications C_{max} and f_{opt} are, for all practical purposes, the same among statistical models, but less similar than among levels of precision. This may be offered as an argument against considering alternative statistical models. But consider the plot of the data in Figure 2; one could draw an average line by eye through the data and arrive at estimates of C_{max} and f_{opt} just as accurate as those estimated by the sophisticated least-squares search technique. The point is that with *good* data most rational statistical procedures should provide similar estimates of C_{max} and f_{opt}. One cannot be certain that this will be so with data of lesser quality or different range. The values of m which determine the shape of the yield curve, on the other hand, are very different between Models 0 and 3. This could have a significant effect on an economic analysis of the yield curve.

In the absence of other criteria for choosing a particular statistical model, the "fit"—least sum of squared residuals—is often selected (Glass, 1969), and is perhaps a reasonable criterion if the goal is interpolation. The goal here is to obtain the best possible parameter estimates in order to make, in essence, extrapolations or predictions. In the latter case the best criterion is not the "fit", but the degree of assumption fulfillment. Statistical Model 3 provided estimates that were least influenced by the addition of error—comparing the parameters' precision between N equalling 1 and 5—inferring the greatest confidence in its estimates. It was also seen from the simulation study that Model 3 best fulfilled the assumptions of a least-squares procedure. Model 3, ironically, "fits" the data the worst, although only by about 6%.

Pienaar and Thomson (1969) have suggested the utilization of an important tool for selecting a statistical model which best fulfills the assumptions of the estimating procedure—residuals examination. Various plots of the residuals suggested by Draper and Smith (1966) were made for the four statistical models (Figure 3). Each statistical model gives a mean residual near zero fulfilling one of the least-squares assumptions (Figure 3A). Plots of residuals against time (Figure 3B) indicate: 1) variation increases with time in Model 0 from 1934 through 1961, violating the assumption of constant residual variance; 2) Model 1 tends to over-correct as there is a propensity for variation to decrease from 1940 through 1967; and 3) Models 2 and 3 are nearly identical in controlling time-oriented variation. Runs—consecutive residuals of the same sign—are evident in all four models, indicating violation of the assumption of residual independence. There are only ten runs in Model 3 giving a probability less than 0.01 that the arrangement of signs is random (Figure 3B). Draper and Smith (1966) suggest, however, that unless the ratio of degrees of freedom to number of observations is small (here 29/34), the effect can be ignored. The dependence of consecutive residuals is undoubtedly due to vitiation of the assumption of no time lags in the fish population. With changes in fishing effort the age structure of the population is altered as well. It might be possible to average out these effects by considering a time period longer than one year, say the average life-span of an individual. That would be about 3 years for a yellowfin tuna, the approximate mean length of the runs. However, that would also reduce the number of observations to eleven and the fishing effort, assumed constant in integration of the model, would vary considerably.

An increase in residual variation with deterministic catch is obvious for Model 0 (Figure 3C), again violating the assumption of constant residual variance. As in the time plot, Model 1 tends to over-correct for the phenomenon exhibited by Model 0. Models 2 and 3 stabilize the variance as might be expected. In the final plot, residuals against fishing effort, the same conclusions may be reached (Figure 3D).

Models 2 and 3 apparently fulfill the assumptions of the least-squares procedure while Models 0 and 1 violate the assumption of constant residual variance. Invoking the previously mentioned criterion for choosing between Models 2 and 3, the best statistical model for this fishery is Model 3.

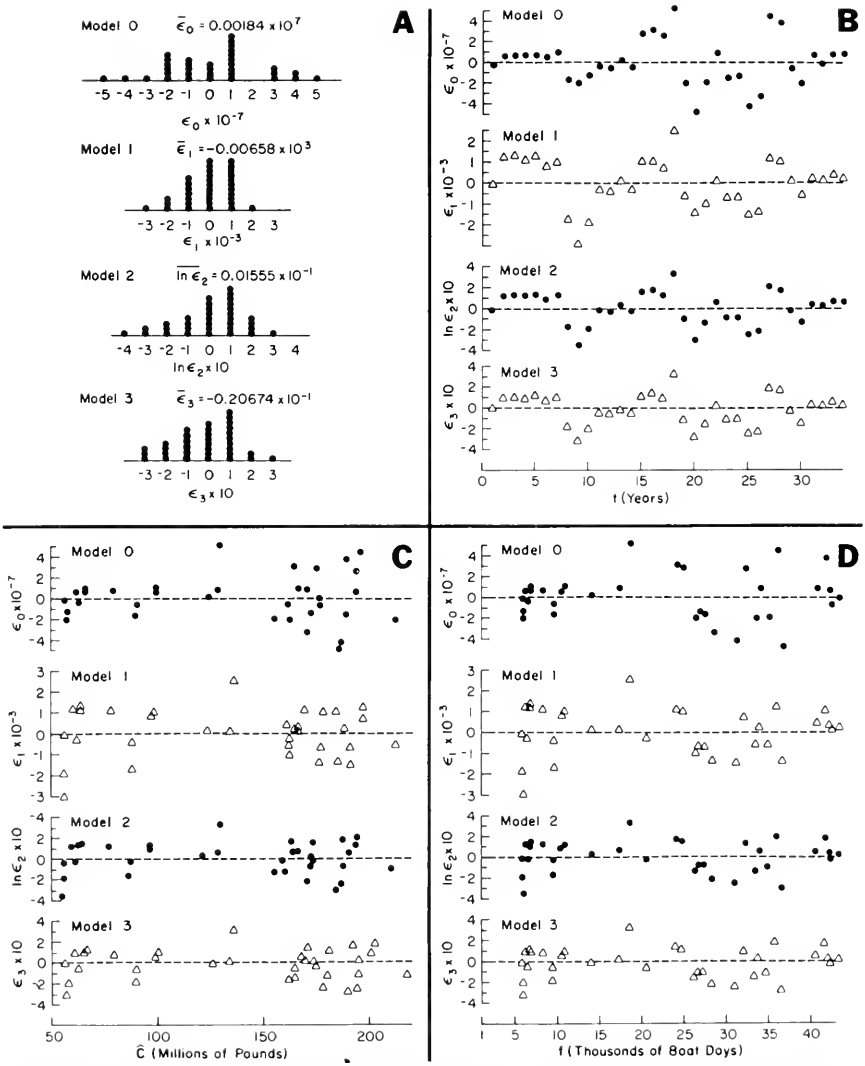

FIGURE 3.—Plots of residuals, ϵ_i, for statistical Models 0, 1, 2, 3, from the generalized production model for the eastern tropical Pacific yellowfin tuna fishery, 1934-67. A. Frequency distributions. B. Residuals against time. C. Residuals against deterministic catch, $\hat{C}_t$. D. Residuals against fishing effort, f_t.

The referee of this paper has raised an important point regarding application of the various statistical models to actual fishery data. In a non-overexploited fishery, generally, the quality and level of catch and effort values increase with time. Relatively speaking, Model 0 in this case places greater weight on more recent data than do Models 1, 2, or 3, and in the absence of any other criteria it might represent the intuitive choice. However, if the quality of the data were a more significant contributor to unequal residence variance than the statistical model, one would expect, in this case, a decrease in the residuals plotted for Model 0 against time, catch, and fishing effort in contrast to the apparent increase for the yellowfin tuna fishery (Figure 3). If one has reason to suspect a significant difference in quality of the data, as would be suggested by a decrease in the residual plots of Model 0, perhaps a solution is to partition the data at the point in time where a significant quality increase occurs. Then fit each set of data individually placing greater weight on the parameter estimates for the more recent set. The specter of the suitability of employing production models over long time periods is also raised by this point. But it is outside the scope of this paper and the reader is referred to the papers cited previously.

SUMMARY

In using a least-squares procedure for estimating parameters of a mathematical model, such as the Pella-Tomlinson technique, there are three assumptions about the residuals for obtaining the best least-squares estimates: 1) the residuals are independent, 2) the residuals have an expected value of zero, and 3) the variance of the residuals is constant (Anscombe and Tukey, 1963; Draper and Smith, 1966; Snedecor and Cochran, 1967). We have observed from the simulation study that two (of four alternative) simple statistical models which are biologically sound—Model 2 (using a logarithmic transformation) and Model 3 (weighting by the inverse of the squared deterministic catch)—fulfill the statistical assumptions for obtaining good least-squares estimates of the generalized production model parameters over a wide range of fishing effort.

On applying these four statistical models in estimating the parameters of the generalized production model for the eastern tropical Pacific yellowfin tuna fishery, residuals examination revealed that the same two statistical models, Models 2 and 3, fulfilled the least-squares estimation assumptions. Models 0 (assumed by Pella and Tomlinson, 1969) and 1 did not. Model 3 was selected as the best model since it involves the direct minimization of the actual residual variance, and is therefore considered to be theoretically superior to Model 2.

Finally, anyone using the generalized production model and the Pella-Tomlinson estimating technique should be aware of, in addition to the proper statistical model, the effect of the value of N in equation (5) on the parameter estimates.

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ADDITIONAL DATA ON THE SPAWNING OF THE HAKE

JOHN S. MACGREGOR[1]

ABSTRACT

In January 1970 samples of hake were taken off southern and central Baja California to study fecundity. In the southern area female hake as small as 130 mm standard length contained developing eggs, and all females longer than 140 mm contained such eggs. There is a marked cline in size at first maturity of hake along the Pacific Coast (hake in the Pacific Northwest exceed 400 mm before reaching maturity). Seventeen female hake 130 to 202 mm long taken in the southern area contained from 3,400 to 19,500 eggs or 229 per gram of fish; 11 females 222 to 305 mm from the central area contained from 3,500 to 110,000 eggs or 243 per gram of fish. In previously published data 22 female hake 346 to 688 mm from northern Baja California contained from 33,000 to 496,000 eggs or 192 eggs per gram of fish. There are no significant differences in fecundity among the three areas. The hake spawns once a year with over 98% of the spawning taking place between January and April.

In 1966, I published data on the fecundity of 22 female hake taken off northern Baja California. In 1970, additional samples of hake were obtained from central and southern Baja California to determine if there were geographic differences in hake fecundity in the offshore waters of Baja California.

The hake samples taken off southern and central Baja California in January contained prespawning females from which estimates of fecundity were obtained.

The hake from northern Baja California for which fecundity data have been published (MacGregor, 1966) appear to be identical to those taken off southern and northern California, while those taken farther to the south are different with respect to growth rate and size at first maturity and, in fact, have been described as a different species (Ginsberg, 1954).

The female hake for which fecundity determinations were made were taken by trawl from the research vessel *David Starr Jordan*. Station J-45-13 at lat 26°07′ N, long 113°07′ W was sampled January 11, 1970. Station J-45-27 at lat 28°44′ N, long 115°15′ W was sampled January 16, 1970. Methods for estimating fecundity of the samples were essentially the same as used previously (MacGregor, 1966).

Previous data on hake fecundity (MacGregor, 1966) were obtained from samples taken by the research vessel *John N. Cobb* (Berry and Perkins, 1966). Station C-58-23 at lat 31°49′ N, long 117°53′ W was sampled March 21, 1963. Station C-58-29 at lat 29°46′ N, long 116°01′ W was sampled March 23, 1963. Station C-58-31 at lat 29°35′ N, long 116°00′ W was sampled March 25, 1963.

FECUNDITY

The range for fecundity data for samples J-45-13 (Table 1) and J-45-27 (Table 2) compare with *Cobb* 1963 samples (MacGregor, 1966) as follows:

	J-45-13	*J-45-27*	Cobb *1963*
Standard length (mm)	130 to 202	222 to 305	346 to 688
Weight (g)	22.1 to 57.0	88.0 to 221.0	300 to 2,750
Gonad weight (g)	0.928 to 4.002	3.279 to 22.710	13.1 to 196.8
Advanced eggs	3,419 to 19,564	3,496 to 110,017	33,000 to 496,000
Eggs per gram of fish	141 to 343	38 to 498	83 to 556

There is no overlap in the ranges of standard length and fish weight of the samples from the three localities. The number of advanced eggs in the ovaries tends to increase with size of fish both within and between samples. However, because of the great variation in the numbers of advanced eggs among the individual fish, there is considerable overlap in gonad weight and numbers of advanced eggs between successive samples.

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TABLE 1.—Fecundity data for 17 female hake taken at Station J-45-13 (lat 26°07′ N, long 113°07′ W) January 11, 1970.

Standard length	Weight	Gonad weight	Gonad index	Advanced eggs		Eggs per gram of fish
				Size range	Number	
mm	*g*	*g*		*mm*		
130	22.1	0.928	4.2	0.50-0.67	3,419	155
131	23.3	1.345	5.8	.57- .73	7,049	303
135	25.2	1.320	5.2	.57- .77	3,564	141
137	22.4	1.518	6.8	.50- .67	6,469	289
137	29.3	1.795	6.1	.53- .70	7,139	244
138	24.8	0.987	4.0	.53- .70	4,048	163
140	28.8	1.350	4.7	.53- .70	4,956	172
140	34.5	2.241	6.6	.57- .77	7,926	230
141	30.5	1.222	4.0	.50- .70	4,534	149
150	21.2	1.198	5.7	.53- .73	4,257	201
150	37.1	2.481	6.7	.53- .73	10,864	293
160	31.5	1.875	6.0	.53- .73	6,447	205
162	30.3	1.957	6.5	.57- .73	7,633	252
170	32.3	2.000	6.2	.50- .70	8,741	271
172	37.7	1.863	4.9	.47- .70	10,731	285
174	37.7	2.000	5.3	.50- .70	7,559	201
202	57.0	4.002	7.0	.50- .67	19,564	343

TABLE 2.—Fecundity data for 11 female hake taken at Station J-45-27 (lat 28°44′ N, long 115°15′ W) January 16, 1970.

Standard length	Weight	Gonad weight	Gonad index	Advanced eggs		Eggs per gram of fish
				Size range	Number	
mm	*g*	*g*		*mm*		
222	100	8.663	9.7	0.50-0.67	29,399	294
230	88	7.011	8.0	.50- .73	23,253	264
233	104	8.488	8.2	.60- .77	29,450	347
238	91	3.279	3.6	.57- .73	3,496	38
245	105	6.292	6.0	.50- .70	17,511	168
247	120	5.956	5.0	.60- .80	18,264	152
252	110	9.341	8.5	.53- .77	43,014	391
268	147	5.308	3.6	.60- .80	13,747	94
275	146	11.860	8.2	.57- .83	38,959	286
287	176	12.972	7.4	.67- .83	24,877	141
305	221	22.710	10.3	57- .80	110,017	498

The mean number of advanced eggs per gram of fish is 229 for sample J-45-13, 243 for sample J-45-27, and 192 for the *Cobb* 1963 samples. Owing to the great range of individual values in each of the samples, there is no significance in the differences between the means. The mean for the 50 fish in the three samples is 216 eggs per gram of fish. The standard error of the mean is 15 eggs or about 7%. In spite of some rather low fecundities, the distribution of eggs per gram of fish approximates a normal distribution indicating that these low counts are within the limits of expected variation.

Eggs/gram	*Percent frequency*
< 50	2
100	24
200	38
300	30
400	2
500	2
>550	2

The low counts apparently did not result from partial spawning of the advanced mode because they were found in fish that were not yet ripe.

RATIOS

To obtain estimates of the numbers of advanced eggs in the hake ovary, first about 100 yolked eggs in the sample were measured in order to delimit the distributions of advanced- and small-yolked eggs. Then the additional advanced-yolked eggs in the weighed sample were counted. An estimate of the numbers of small eggs was obtained from the ratio of large to small eggs in the measured frequency distribution, but because this estimate is based on relatively few eggs it is less accurate than the estimates of advanced eggs. Estimates of the numbers of small eggs per gram of fish ranged from

33 to 766 and averaged 248. This compares with 38 to 556 with an average of 216 for the advanced eggs for all samples.

The correlation obtained by MacGregor (1966) between percentage of eggs in the advanced mode and advanced eggs per gram of fish has no mathematical significance because both variables are related. Actually there is no relation between the number of advanced eggs and the number of small eggs. Ovaries containing either high or low numbers of advanced eggs per gram of fish may contain either high or low numbers of small-yolked eggs. However, the conclusion that the great variation in the ratios of large to small eggs makes multiple spawning unlikely seems to be valid.

Of 366,093 hake larvae taken on monthly cruises in the 6 years 1951 through 1956, 87% were taken in January, February, and March, 11.5% in April, and the remaining 1.5% in the remaining 8 months. Several large samples of hake taken off California in April 1970 showed that the advanced eggs were no longer present in the ovaries of the females while the smaller yolked eggs were. Because there is no evidence of further egg development in the ovaries and no evidence of heavy spawning subsequent to April in the plankton, we must assume that these small-yolked eggs are resorbed following spawning of the advanced mode.

SIZE AT FIRST MATURITY

One hundred thirty-six hake from sample J-45-13 (southern Baja California) were examined for stage of sexual maturity. Eighty hake from sample J-45-27 (central Baja California) and an additional seven hake taken in 1961 near Cedros Island (central Baja California) were also examined (Table 3).

In sample J-45-13 the largest immature male was 127 mm in length, and the largest immature female 138 mm. The smallest maturing male was 119 mm, and the smallest maturing female 125 mm. All males 129 mm and longer and all females 140 mm and longer were maturing.

In sample J-45-27 the range of fish length was not as good for determining size at first maturity. The smallest maturing male was 137 mm long, and it appeared that all males 159 mm and longer were mature. The smallest mature female was 222 mm in length, and all females of this length and longer were maturing. However, on the basis of the 1961 sample it appears that all females were maturing at some length between 202 and 222 mm.

It is difficult to determine the size at first maturity for hake off northern Baja California and California because very few fish of suitable size, taken during the spawning season, were available. For off-season hake the gonad index may

TABLE 3.—Size at first maturity. Southern Baja California, sample J-45-13, lat 27°07′ N, long 113°07′ W, January 11, 1970. Central Baja California (Cedros Island area), sample J-45-27, lat 28°44′ N, long 115°15′ W, January 16, 1970, and sample B-6111-3, Cedros Island, November 25, 1961. Males judged mature or immature by size and appearance of testes. Females judged immature if largest eggs in ovary were 0.20 mm or less in diameter (not yolked); probably maturing if maximum egg diameter was 0.23 to 0.47 mm; mature if maximum egg diameter was 0.50 mm or larger. Immature, not sexed: gonads not developed enough so that sex can be determined by gross examination.

Sample	Males			Females				Not sexed	
	Standard length	Immature	Mature	Standard length	Maximum egg diameter (mm) 0.0-0.10	0.30-0.47	0.50-0.77	Standard length	Immature
	mm			*mm*				*mm*	
J-45-13	119-127	6	6	122-124	6	0	0	100-118	19
				125-128	8	1	0		
	129-166	0	50	130-138	9	4	6		
				140-202	0	0	21		
					Maximum egg diameter (mm) 0.0-0.20	0.37	0.67-0.83		
J-45-27	137-140	1	1	172-197	3	0	0	120-138	42
	159-307	0	21	222-228	0	1	1		
				230-305	0	0	10		
B-6111-3				236	0	0	1	148-202	6

indicate maturity. Generally, hake with gonad indices of less than 0.5 (i.e., the gonad weight is less than 0.5% of the fish weight) do not contain yolked eggs in their ovaries. There is no yolk in eggs up to 0.20 mm diameter, and fish containing such eggs a few months before or after the spawning season may be considered immature. Eggs in an ovary having a gonad index of 0.8 have a maximum diameter of about 0.34 mm and at a gonad index of 1.5, about 0.43 mm maximum diameter. The eggs generally are not large enough to count (over 0.65 mm diameter for the largest eggs in the ovary) until the gonad index is about 3.5, and then only for fish having a fecundity of less than 100 eggs per gram of fish. If we apply the same criterion (a gonad index of less than 0.5 as indicating an immature fish) to the males, we can roughly estimate the size at maturity for off-season hake.

Applying this criterion of maturity to a number of miscellaneous hake samples taken in the off-season for spawning off northern Baja California and southern California, it appears that all males 285 mm and longer and all females 340 mm and longer were mature.

Best (1963) estimated that all hake, both males and females, taken off northern California were mature at 400 mm total length (about 360 mm standard length). The length at which all fish are mature could be somewhat less as he had a limited number of smaller fish in his samples.

Nelson and Larkins (1970) found that all fish of 450 mm total length (about 405 mm standard length) were mature in the Pacific Northwest. Apparently they also had few smaller fish to work with, and the length at which all fish are mature could be somewhat less.

DISCUSSION

The mean number of eggs per gram of fish was not significantly different among the three samples, J-45-13, J-45-27, and *Cobb* 1963. These three samples were taken in widely separated localities, and although they were similar with respect to relative fecundity, questions have been raised as to the distinctness of the north Pacific hake with respect to race or even species.

Ginsberg (1954) assigned the north Pacific hake to two species based on morphometric and meristic characters. His descriptions were based on 12 specimens of *Merluccius productus* taken off Washington, Oregon, and California, as far south as San Diego, and eight specimens of *M. angustimanus* taken in the Gulf of Panama, the Gulf of California, off the Pacific Coast of Baja California, and off Del Mar, Calif.

Ahlstrom and Counts (1955) could find no evidence of more than one species of hake in their extensive collections of eggs and larvae taken between San Francisco and the southern tip of Baja California. All of the small fish that had fully developed dorsal and anal fins had fin ray counts that fell within the range of *M. productus* but outside of the range of *M. angustimanus* as given by Ginsberg.

F. H. Berry (unpublished data) studied numerous additional specimens of hake from Baja California and California. He concluded that *M. productus* and *M. angustimanus* were the same species and the differences in meristic and morphometric characters, used by Ginsberg to separate the species represented a latitudinal cline.

There was certainly a marked cline in size at maturity for the hake used in this study, especially when these data are compared with data given by other authors. The size at which all fish were mature was as follows:

	Sex	Standard length
Southern Baja California	males	129 mm
	females	140 mm
Central Baja California	males	159 mm
	females	202-222 mm
Northern Baja California and southern California	males	285 mm
	females	340 mm
Northern California (Best, 1963)	both	360 mm
Pacific Northwest (Nelson and Larkins, 1970)	both	405 mm

The differences between areas are so great that the roughness of some of the estimates does not affect the conclusion that these differences are very real.

The distribution and growth patterns of the European hake, *M. merluccius*, is similar to that of *M. productus* in many ways. The European hake ranges from Norway to at least Mauritania in Africa (Hart, 1948) while *M. productus*

ranges from Alaska to at least the southern tip of Baja California. Both species grow to much larger sizes in the northern parts of their ranges, but are much smaller in their southern ranges. *M. merluccius* in the Mediterranean Sea is smaller than the north Atlantic form, and both the southern Baja California and African coasts apparently produce dwarf races of their respective hake species.

Most recent information from the Guinean Trawling Survey (Williams, 1968) shows that there are continuous populations of hake from Norway to South Africa. However, the hake taken off the west coast of Africa are ascribed to several species other than *M. merluccius*. The West African hake, *M. polli* and unidentified *M.* spp. were taken throughout the survey area from the Gambia border to the Congo. The Senegal hake *M. senegalensis* was taken in the northern areas between the Gambia border and southern Liberia with one questionable record from Nigeria, and the South African hake *M. capensis* was taken in the southern areas between Cameroun and the Congo. A sample of 50 *M. senegalensis* averaged 26.3 cm total length (range 18 to 27), and a sample of *M. polli* averaged 41.7 cm total length (range 35 to 49).

SUMMARY

The north Pacific hake, *Merluccius productus*, ranges from Alaska to at least southern Baja California.

The fecundity of individual hake varied greatly off Baja California and southern California, but there was no significant difference in average fecundity among the samples taken from widely separated sampling stations in this area. Estimates of the number of advanced eggs contained in 50 prespawning hake averaged 216 eggs per gram of fish.

Average size at first maturity for female hake varied from 133 mm standard length off southern Baja California to about 340 mm off northern Baja California and southern California. Males appeared to mature at smaller sizes 128 mm in the south to 285 mm in the north.

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THE LOW-TEMPERATURE THRESHOLD FOR PINK SALMON EGGS IN RELATION TO A PROPOSED HYDROELECTRIC INSTALLATION

JACK E. BAILEY[1] AND DALE R. EVANS[2]

ABSTRACT

A proposed hydroelectric installation in southeastern Alaska would alter the seasonal pattern of stream temperatures and pose a threat to the natural production of pink salmon, *Oncorhynchus gorbuscha*. Analysis of experiments reported in the literature indicated that such an installation might lower stream temperatures below the threshold normal for the embryonic development of pink salmon. Our experiments with pink salmon eggs incubated in refrigerated water showed that the eggs required initial temperatures above 4.5° C for normal embryonic development. An increase in mortalities and in alevins with spinal deformities occurred when initial incubation temperatures were 4.5° C and lower; initial incubation at 2° C resulted in complete mortality. The proposed hydroelectric installation could result in temperatures as low as 4.5° C during spawning and initial incubation and could therefore be expected to cause an increase in mortality and the occurrence of deformed alevins. The low temperature would be followed by higher than normal winter incubation temperatures, which would have an unknown effect on the time of emergence of fry. A tunnel intake designed to draw water of a desirable temperature on demand would be required to protect salmon.

In 1964 the Bureau of Reclamation (now the Alaska Power Administration) started feasibility studies on a hydroelectric installation on Lake Grace, 51 km northeast of Ketchikan, Alaska. Grace Creek, the lake's outlet stream, enters the sea 4 km from Lake Grace. An impassable falls 2 km above tide water prevents migrating fish from reaching Lake Grace. Below the falls, the creek provides important spawning, incubation, and rearing areas for salmonids, especially pink salmon, *Oncorhynchus gorbuscha*.

The proposed dam would divert practically all of the water from Grace Creek through a hydroelectric plant and back into Grace Creek about 1.2 km from tide water. Because of the design of the dam and of the water intake, the temperature of the lower 1.2 km of the creek could be changed to lower than normal in summer and fall and higher than normal in winter.

Analysis of the studies by Combs and Burrows (1957) and Combs (1965) on the relation between temperature and the development of salmonid embryos indicated that when the hydroelectric facility is constructed, water temperatures in the principal spawning areas of Grace Creek might be too low for normal embryonic development. We therefore estimated the temperatures likely to occur in Grace Creek. Because these temperatures seemed critically low, we conducted laboratory experiments to determine precisely the low-temperature threshold or minimum temperature for the normal development of embryos of pink salmon, the major species in Grace Creek.

In this report we analyze the effects of the proposed installation on the temperature regime of Lake Grace and Grace Creek and describe the threshold temperatures for development of pink salmon embryos, and then relate the two studies and discuss their implications.

EFFECTS OF PROPOSED INSTALLATION ON TEMPERATURE REGIME OF LAKE GRACE AND GRACE CREEK

To consider the effects of the installation on the temperature regime, we compared the seasonal temperature pattern of Grace Creek under normal conditions with the temperatures likely

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to occur when the proposed power plant is in operation.

The daily maximum and minimum temperatures for Grace Creek (U.S. Geological Survey, 1966: 15; 1967: 17; 1968: 16) for the first and second half of each month between April 16, 1965, and March 31, 1967, were averaged to determine the annual temperature pattern under normal conditions (Figure 1). The highest temperature for this period was 17.8° C and the lowest was 0.6° C. Normally, Grace Creek temperatures reach a maximum of about 15° C at the start of the spawning season in mid-August, and decline to 10° C at the end of the spawning season the first week of October. After the spawning season, stream temperatures continue to fall, reaching about 6° C by late November and about 1.5° C in midwinter, when ice cover forms on Lake Grace during most years. The average stream temperature is about 1.5° C from the end of December until the end of March.

If the proposed power development on Lake Grace is completed, it would significantly change the physical dimensions and waterflow of the lake. The dam at the outlet of Lake Grace would raise the lake surface from the present elevation of 131.1 m (mean sea level) to a maximum elevation of 152.4 m and increase its surface area from 668 to 1,046 ha. Water would be diverted through a pressure tunnel and exposed penstock to a powerhouse on Grace Creek, 1.21 km downstream from the dam. The intake elevation of the diversion tunnel would be constant at 125.0 m, but the elevation of the reservoir surface would change from 131.4 to 152.4 m as the active reservoir capacity of 1.84×10^8 m^3 is used. The maximum depth of the natural lake is 129.5 m.

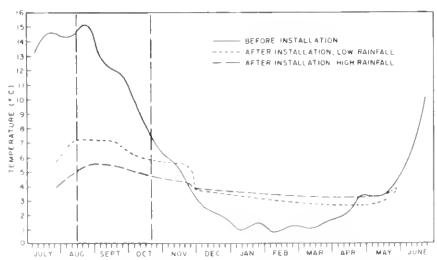

FIGURE 1.—Average annual temperature pattern of Grace Creek, based on temperature records from April 16, 1965, through March 31, 1967, and temperature patterns that might prevail during years of extreme high and low rainfall if water is drawn from a reservoir at the 125.0-m elevation.

Most of the water in the stream below the powerhouse would come through the powerhouse and would therefore be similar in temperature to the water at the tunnel intake level of the lake. To predict the temperature of this water, we obtained temperature-depth profiles from Lake Grace during the freshwater phase of the reproductive cycle of pink salmon. Profiles were taken on July 27, 1961, August 12, 1965, September 16, 1965, October 8, 1965, November 18, 1965, and March 25, 1965 (Figure 2). In addition to the temperature-depth profiles, we obtained thermograph records which indicated surface water temperatures attained 4° C November 28, 1965, and again May 28, 1966. These thermograph records provide our best estimate of the dates of autumn and spring overturn of Lake Grace. Although these data were not all taken during a single reproductive cycle of pink salmon, we feel they are representative. These predicted estimated temperatures of the lake waters that would enter the intake are probably higher than would actually occur because increasing the depth of the lake increases its thermal capacity and results in colder deep water (Hutchinson, 1957). No correction was made for the cooling effect of deeper water.

The actual surface elevation of the reservoir and therefore the depth of the tunnel intake would depend on operational requirements of the power plant and the flow of water into Lake Grace. The fixed tunnel intake at the 125.0-m elevation could be under 6.4 to 27.4 m of water because the proposed active reservoir elevation varies from 131.4 to 152.4 m. We allowed for these fluctuations in using the temperature-depth profiles as an indication of water temperatures at tunnel intake depth.

The project development plan for Lake Grace included a graphic model of simulated reservoir water surface elevations for each month from

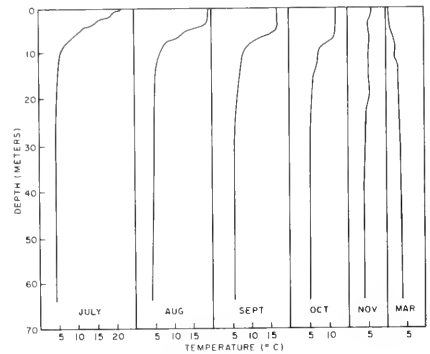

FIGURE 2.—Temperature-depth profiles from Lake Grace during months of the freshwater reproductive cycle of pink salmon.

January 1928 through December 1964.[3] The basis of the model included records of streamflow and climate from Grace Creek and several nearby streams and also a computer study of the monthly operation of the power plant. We have chosen the year of lowest water level (1948) and the year of highest water level (1934) from the surface elevation model to illustrate the range of temperature patterns that might be caused by fluctuating water levels (Figure 1). The simulated water levels and depth of water above the entrance to the intake tunnel at 125.0 m used to predict water temperatures are shown in Table 1.

In Grace Creek, the probable result of the power plant would be lower than normal temperatures during summer and fall when salmon eggs are beginning their development and higher than normal temperatures during the winter when eggs and alevins are completing their development. Temperatures of Grace Creek during the normal spawning season currently range from 15° to 10° C, but under the conditions of power plant operation, temperatures would be from 7° to 5° C. During the first month after spawning, temperatures normally range from 10° to 6° C, but under the altered conditions they may be reduced to only 6° to 4.5° C. The predicted temperatures for the winter incubation period, 3° to 4° C, would be consistently higher than the 1° to 3° C in the unaltered stream.

[3] Alaska Power Administration. 1968. Lake Grace Project, Alaska. On file, Alaska Power Administration, Federal Building, Juneau, Alaska 99801.

LOW-TEMPERATURE THRESHOLD FOR NORMAL DEVELOPMENT OF EMBRYOS

Several workers have studied the low-temperature threshold for normal development of embryos of fishes. In this section we review their findings and describe our laboratory experiments with pink salmon.

EXPERIMENTS BY OTHER WORKERS

An important aspect of the effects of low temperatures is the stage of development at the time the critical temperature is imposed on the embryo. Combs and Burrows (1957) associated high mortalities and gross anomalies in embryos of chinook salmon with low temperatures during the pregastrula stages; they used a significant rise in mortality in defining the low-temperature threshold. Combs (1965), Efimov (1962), and Price (1940) found that salmonid eggs were most sensitive to low temperature in the blastula and early gastrula stages. These authors demonstrated that once gastrulation is well underway, the embryos can tolerate temperatures close to freezing.

TABLE 1.—Estimated water temperatures that would have prevailed at the 125.0-m elevation (mean sea level) of the proposed tunnel intake of Lake Grace if the proposed power plant had been in operation during the year of lowest water level (1948) and the year of highest water level (1934) from 1928 to 1964.

	Water surface elevation (m)		Depth of water above tunnel intake (m)		Water temperature at tunnel intake (° C)	
Month	1948	1934	1948	1934	1948	1934
July	135.6	152.4	10.4	27.4	5.6	3.9
August	134.1	152.4	9.1	27.4	7.2	5.0
September	137.2	150.9	12.2	25.9	7.2	5.6
October	141.7	152.4	16.8	27.4	6.1	5.0
November	143.2	152.4	18.3	27.4	5.6	4.4
March	135.6	149.4	10.7	24.4	2.8	3.3

Adverse effects of low temperatures during certain stages of development were also observed for other fishes. Kinne and Kinne (1962) exposed embryos of the cyprinodont *Cyprinodon macularis* to different temperature-salinity-oxygen combinations and found a period of "low thermal stability," which we presume to mean low resistance, in embryos exposed to critically low temperatures during early development (fertilization to gastrulation). Stockard (1921) conducted a number of experiments with eggs of the cyprinodont *Fundulus heteroclitus* which he placed in a refrigerator at 5°, 7°, and 9° C for various lengths of time and at various stages of development. Development was almost, if not completely, stopped at 5° C and greatly slowed at 9° C. Exposure to 5° C just after gastrulation commenced was not noticeably injurious, but exposure to low temperatures during earlier stages resulted in increased mortalities and gross anomalies among survivors.

Piavis (1961) incubated sea lamprey eggs at various constant temperatures and learned that viable burrowing larvae could be produced at 15.6° C but not at 12.8° C. McCauley (1963) also explored the lethal temperature limits of embryonic sea lamprey. He found that the narrow range of constant temperature, 15.0° to 25.0° C, necessary for successful hatching may be extended to 12.2° to 25.6° C if gastrulation is completed before the eggs encounter temperature extremes.

The work of Täning (1952) on the effects of temperature on the development of *Salmo trutta trutta* supports Stockard's conclusion that the earlier the stage of development is arrested, the more severe will be the effect.

The lowering of temperatures in Grace Creek by the proposed hydroelectric plant would be greatest just before and during gastrulation of the pink salmon embryos (Figure 1).

EXPERIMENTS IN LABORATORY ON PINK SALMON

We conducted an experiment in the laboratory to determine if pink salmon eggs could survive and develop normally under the projected thermal regime for Grace Creek. The eggs for the study came from two pink salmon collected September 7, 1966, from Grace Creek. The eggs were thoroughly mixed and fertilized by sperm from two males in the field.

Embryonic development had begun before the eggs were placed in the experimental array because the temperature in the transporting container ranged from 7° to 12° C (average, 10.8° C) during the 10-hr trip to the laboratory. According to Soin (1954) the first cleavage division occurs in pink salmon eggs about 7 hr after fertilization at 11° C. Knight (1963) showed that about 2.5 hr elapse between successive cleavage divisions in rainbow trout eggs at 12.2° C. Therefore, we estimate, but did not confirm, that the Grace Creek pink salmon eggs completed two cleavage divisions before they were transferred to the controlled temperatures of the experiment.

The eggs were incubated in 55- and 42.5-mm diameter Buchner funnels with perforated plates. Each of the large funnels was stocked with 120 eggs and each of the small ones with 25 eggs. The water was introduced through the stem of the funnel to produce an upwelling flow through the plates that supported the eggs. The water was not recirculated, and flow rates[4] were set to deliver an apparent velocity of about 200 cm hr to the eggs. Dissolved oxygen content of the water as it entered the funnels was above 8 ppm at all times.

The experiment involved 16 treatments consisting of four initial incubation temperatures each with four exposure periods. The four temperatures were ambient,[5] 4.5°, 3.0°, and 2.0° C; and the four exposure periods were 15, 27, 37, and 103 days. All of the exposure periods began 10 hr after fertilization on September 7, 1966. When the experimental cold treatment for each lot was completed, the eggs were transferred to ambient temperature to complete their incubation. Temperatures were recorded continuously; the daily means (Figure 3) were usually within ±0.5° C of the planned levels. In-

[4] Apparent velocity was obtained by dividing the rate of flow to the egg container in cubic centimeters per hour by the cross-sectional area of the container in square centimeters.

[5] Ambient temperature is the unmodified temperature of the laboratory water supply. See Figure 3.

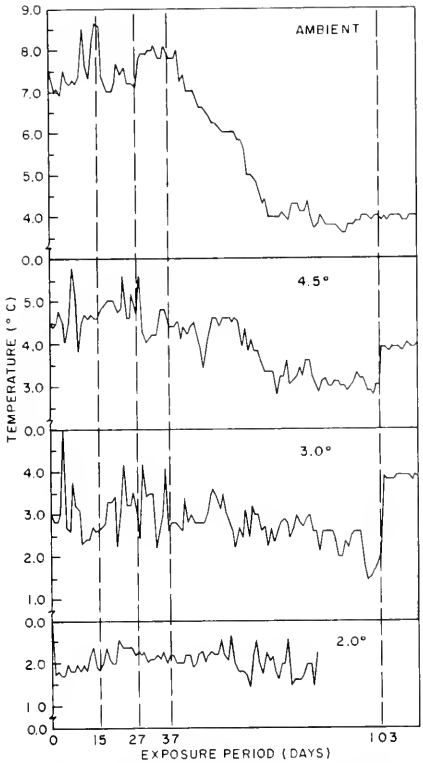

FIGURE 3.—Four initial incubation temperatures to which pink salmon eggs were subjected for four exposure periods in laboratory experiments on effects of low temperature on growth and development. Dashed lines indicate when individual lots were transferred to ambient temperature (see footnote 5).

frequent variations as great as ±2° C for 1 day were observed. Mean temperatures for the 37-day period September 7 through October 14 were within ±0.2° C of the planned levels. Ambient temperature, initially 6.9° to 8.7° C, dropped gradually to the usual winter level of 4.0° C by mid-November.

We made no direct observations of the stages of development of the embryos when they were transferred to ambient temperature (on days 15, 27, 37, and 103), but in a separate experiment we observed development of pink salmon embryos in relation to temperature. At temperatures approximating ambient in the present experiment, the eggs began gastrulation about the 10th day and completed gastrulation about the 26th day. Eggs incubated initially at 4.5° C began gastrulation about the 21st day and completed gastrulation about the 45th day. Eggs incubated initially at 3.0° C began gastrulation about the 34th day and completed gastrulation about the 62d day.

We controlled water temperatures during incubation by mixing chilled and unchilled water in the intake line to each incubation funnel. A continuous flow of fresh water chilled to 1.0° ±0.3° C was obtained by operation of a 1/3-hp refrigeration unit. The cooling coils and agitator propeller were suspended in an insulated 20-gal fiberglass tank. Unchilled or ambient water was introduced through Y fittings to produce the required temperature for experimental lots of eggs.

Eggs incubated entirely at ambient temperature were first to hatch—the midpoint of hatching occurred December 9, 1966, 94 days after fertilization. The last eggs to hatch were from the group that was incubated initially at 3.0° C for 103 days. The survivors of the prolonged cold treatment hatched February 8, 1967, 154 days after fertilization.

Mortalities were inversely related to initial incubation temperatures. None of the eggs incubated at 2.0° C survived (Table 2); at 3.0° C, about 75% died; and at 4.5° C, about 10% died. Average mortality of eggs incubated entirely at ambient temperature was only 3%.

The occurrence of developmental anomalies was also associated with severity of the initial cold treatments. No alevins were produced in the 2° C treatment lots. Mild spinal deformities, various degrees of spinal curvature in the vertical plane, occurred in the 3.0° C and 4.5° C lots. The spinal flexures were not always severe enough to be easily recognized as deformities, but the deformity caused the lengths of alevins in these lots to be less uniform than the lengths of alevins in the ambient temperature lots.

TABLE 2.—Percentage mortality from fertilization to hatching of pink salmon in relation to initial incubation at low temperatures (number of eggs in each lot in parentheses).

Exposure period (days)	Percent mortality at temperature treatment[1]			
	Ambient	4.5° C	3.0° C	2.0° C
15	4 (24)	12 (24)	71 (24)	100 (24)
27	0 (25)	12 (25)	77 (22)	100 (25)
37	5 (20)	10 (21)	60 (20)	100 (21)
103	2 (41)	7 (41)	82 (40)	100 (40)

[1] See Figure 3 for temperature regime for each treatment.

Range in lengths among alevins in the ambient temperature lots was 20.9 to 22.9 mm, but range in length in the 3.0° C lots was 15.1 to 22.3 mm (Table 3).

Because of the increased mortality and abnormal embryonic development of Grace Creek pink salmon eggs at temperatures of 4.5° C and lower, we conclude that initial incubation temperature for these eggs should be higher than 4.5° C. This is in agreement with the 4.4° to 5.9° C threshold for normal development of sockeye and chinook salmon eggs found by Combs (1965).

TABLE 3.—Ranges in lengths (millimeters) of alevins from eggs treated at four temperatures (number of alevins measured in parentheses).

Exposure period (days)	Ranges in total length (mm) at—			
	Ambient temperature	4.5° C	3.0° C	2.0° C
15	21.3-22.6 (10)	20.8-23.2 (10)	18.4-21.9 (7)	--
27	21.1-22.8 (10)	21.0-23.1 (10)	20.5-21.7 (5)	--
37	20.9-22.3 (10)	19.3-22.3 (10)	19.4-22.3 (8)	--
103	21.0-22.9 (10)	20.2-22.4 (10)	15.1-20.3 (7)	--

DISCUSSION

The proposed Grace Creek hydroelectric power plant focuses attention on a fishery problem that may become increasingly important if more hydroelectric plants are to be built on Alaska streams. Where water for power generation is drawn only from the deeper and colder waters of reservoirs, the resulting stream temperatures would be lower than normal during the salmon spawning season and initial incubation period (Figure 1).

At Grace Creek the expected changes in water temperature could affect salmon in several ways. Delay in ripening of gonads of the adults after they enter the streams because of the lowered temperatures (Reingold, 1968) might result in late spawning. The low initial incubation temperatures would further delay development of embryos, but if normal cleavage were not disrupted this delay could be offset by the higher winter temperatures. The net effect on time of emergence and seaward migration of the fry is not known. The predicted initial incubation temperature of 4.5° to 7.2° C (Figure 1) for pink salmon eggs at Grace Creek includes the temperature 4.5° C, at which we detected abnormal development and increased mortality of the embryos. Measures should therefore be adopted to prevent deleterious temperature changes. Provision of an intake designed to draw water of a desirable temperature on demand is suggested as minimum action to protect the salmon.

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PRIMARY PRODUCTION IN THE MID-SUBARCTIC PACIFIC REGION, 1966-68

JERRY D. LARRANCE[1]

ABSTRACT

Primary productivity, chlorophyll *a*, net zooplankton, nutrients, and associated physical variables were measured on seven cruises in the mid-Subarctic Pacific Region in 1966-68. Most of the data were collected between lat 46° N and the central Aleutian Islands, although several measurements were made as far south as lat 40° N. Primary productivity and chlorophyll were higher in Aleutian coastal waters than in areas to the south, but no other major differences among upper zone domains were consistent seasonally. Production was low in winter, high in spring, and intermediate throughout the summer. Annual productivity was between 80 and 100 g C/m^2. Chlorophyll *a* concentrations changed only slightly except in March when chlorophyll was high during the early part of the phytoplankton bloom.

Low light intensities limited primary production during the winter, and zooplankton grazing appeared to limit production in summer and part of spring. Nutrients and light were always sufficient to support high productivity during spring and summer except in late summer when some nutrients, particularly nitrate, were very low south of lat 44° N; however, the productivity did not appear severely limited. The main source of phosphate replenishment in the upper layers during spring and summer was probably in situ regeneration by zooplankton rather than upwelled deep water.

The pelagic biota of the Subarctic Pacific Region has long been recognized as distinct from that in the Subtropical Region, and the Subarctic is thought to be generally more productive. Until the introduction of the carbon-14 technique by Steemann Nielsen (1952), however, no adequate means existed for directly measuring primary productivity in the open ocean. Since that time thousands of measurements of primary production have been made throughout the Tropical and Subtropical North Pacific. Measurements in the Subarctic Pacific have been fewer and more localized.

Koblents-Mishke (1965), who summarized data from the Pacific Ocean, estimated that primary productivity in the mid-Subarctic Region averaged about 150 to 250 mg C/m^2 per day or 55 to 91 g C/m^2 per year. She estimated average production in the Gulf of Alaska and along the Washington and Oregon coasts to be between 250 and 650 mg C/m^2 per day (90-240 g C/m^2 per year) and in the transition area of the southern Subarctic to be 100 to 150 mg C/m^2 per day (35-55 g C/m^2 per year). As discussed by Koblents-Mishke, these estimates are rather imprecise because productivity at most of the stations was measured only at the surface and not throughout the euphotic zone and because many of the measurements were made in artificial light of various intensities. Comprehensive analyses of annual cycles were seldom possible because surveys have been made during all seasons in only a few studies.

From detailed year-round surveys, Anderson (in press) estimated annual primary production in oceanic waters off Washington and Oregon to be 125 g C/m^2. Mean annual primary production at Ocean Station "P" (lat 50° N, long 145° W) in the Gulf of Alaska was 48 g C/m^2 in 1960-66 (McAllister, 1969). Although primary productivity has been measured on several individual cruises through the region (McGary and Graham, 1960; Faculty of Fisheries, Hokkaido University, 1961; Doty, 1964; Koblents-Mishke, 1965), no previous time-series studies of productivity have been made in the central Subarctic Region west of Station "P."

Primary productivity, zooplankton abundance, and related physical and chemical oceanographic

[1] National Marine Fisheries Service, Biological Laboratory, Seattle, Wash. 98102.

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variables were measured on several cruises in 1966-68 within the Subarctic Region in conjunction with studies of abundance and distribution of Pacific salmon (genus *Oncorhynchus*). Productivity data are listed in Larrance (1971), zooplankton is discussed by Day (1970),[2] and physical data are listed in Ingraham and Fisk (1970). The objectives were to obtain an estimate of annual productivity and to detect what differences in levels of productivity, if any, occurred among several oceanographic areas identifiable by physical characteristics.

METHODS

Primary productivity and related variables were measured on cruises of the RV *George B. Kelez* in March, June, and September 1966; January-February, June-July, and August 1967; and May 1968; and on a cruise of the MV *Paragon* in June 1966 (Table 1). Measurements in 1966-67 were south of Adak Island (long 176°25′ W) except in January-February 1967 when the cruise track was along long 162° W and in May 1968 when it was along long 165° W (Figure 1). Primary productivity was measured by the carbon-14 method introduced by Steemann Nielsen (1952) and modified by Strickland and Parsons (1965). Productivity stations were normally taken shortly before dawn and local apparent noon (LAN); incubation periods were for one-half the daylight period, i.e., from dawn to LAN and from LAN until about twilight. Seawater was sampled with 6-liter plastic water bottles at depths determined from the penetration of light below the sea surface. These "light depths" were 100, 61, 35, 18, and 3% of the surface intensity according to the fractions of light transmitted by neutral-light filters used in the productivity incubations. The depths

[2] Day, D. S. 1970. Distribution of zooplankton from the mid-Subarctic Region of the Pacific Ocean, 1966-67. Natl. Mar. Fish. Serv., Biol. Lab., Seattle, Wash. Unpubl. manuscr.

TABLE 1.—Summary of areas, dates, and stations on which primary productivity was measured, 1966-68[1].

Cruise no.	Vessel	Dates	Number of stations	Area
		1966		
K1-66	*Kelez*	March 18-28	Productivity - 6 Chlorophyll and nutrients - 9 Total - 9	Adak Is. to lat 41° N
P2-66	*Paragon*	June 10-21	Productivity - 8 Chlorophyll and nutrients - 10 Total - 10	Adak Is. to lat 41° N
K3-66	*Kelez*	Sept. 8-20	Productivity - 11 Chlorophyll and nutrients - 19 Surface productivity - 14 Total - 28	Adak Is. to lat 40° N
		1967		
K1-67	*Kelez*	Jan. 30-Feb. 15	Productivity - 10 Total - 10	Along long. 162° W between lat 54° and 46° N
K5-67	*Kelez*	June 24-July 3	Productivity - 10 Total - 10	Adak Is. to lat 46° N
[2]K6-67	*Kelez*	July 8-July 11	Productivity (½ day only) - 4	Alaskan Stream
K7-67	*Kelez*	Aug. 21-Aug. 28	Productivity - 7 Total - 7	Adak Is. to lat 46° N
		1968		
K2-68	*Kelez*	May 9-15	Chlorophyll and nutrients - 5 Total - 5	Along long. 164° W between lat 53° and 49° N

[1] Only portions of cruises discussed in this report are included in Table 1.
[2] Data from cruise 6-67 were averaged with data from station 5-67 for this report.

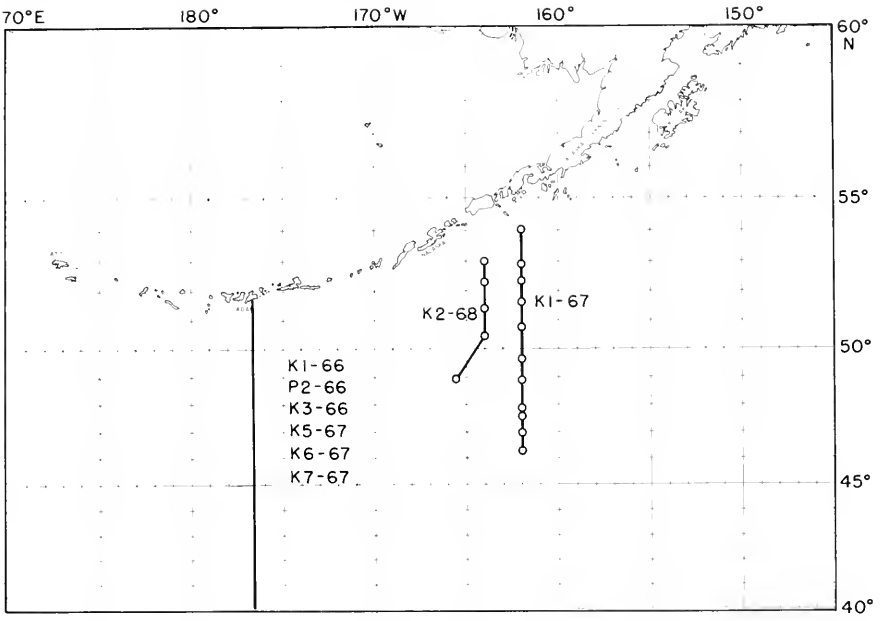


FIGURE 1.—Tracks of Bureau of Commercial Fisheries cruises in 1966-68 in the Subarctic Pacific Region during which primary productivity was measured.

were calculated from Secchi disk readings converted to extinction coefficients (Poole and Atkins, 1929). Sampling depths of the morning stations were computed from the preceding day's Secchi-disk readings. Duplicate light-bottle samples under the neutral-light filters were incubated on deck in daylight and cooled with running sea water. After incubation, samples were filtered through Millipore[3] filters, pore size 0.45 μ, for radioassay.

Stock solutions of $Na_2{}^{14}CO_3$ were prepared according to Strickland and Parsons (1965) but were standardized by using liquid-scintillation techniques. A 1.00-ml portion from an ampoule containing the stock carbonate solution was introduced in 10 ml of a suitable phosphor solution, and its count rate determined in a Packard Tri-Carb[4] scintillation spectrometer. One liter of phosphor solution contained 800 ml toluene, 200 ml Sterox[5] (a surfactant required to make the water miscible in toluene), 5.0 g PPO (2, 5-diphenyl-oxazole), and 0.3 g bis-MSB (p-bis-(o-methylstyrl)-benzene). Counts of an external radium standard were also recorded and the absolute activity (dpm) was determined from a quench correction curve relating efficiency to the count rate of the external standard (Wang and Willis, 1965). Although scintillation counting

[3] Millipore Corp., Ashby Rd., Bedford, Mass. 01730. References to trade names in this publication do not imply endorsement of commercial products by the National Marine Fisheries Service.

[4] Packard Instrument Co., Inc., 2200 Warrenville Rd., Downers Grove, Ill. 60515.

[5] Jefferson Chemical Co., P.O. Box 53300, Houston, Tex.

is more efficient than planchet counting, productivity samples were routinely counted on planchets in a gas-flow geiger detector for convenience. The efficiency of the geiger counter was determined by counting a standard source of known activity so that absolute activity of the samples could be related to those of the stock solution.

Samples for chlorophyll *a*, phosphate, silicate, and nitrate-nitrite were also drawn from the water bottles. Nutrient samples were frozen in plastic bottles and returned to Seattle for analysis. Phosphate and silicate concentrations were determined by methods given by Strickland and Parsons (1965), and nitrate-nitrite samples were analyzed by the method of Wood, Armstrong, and Richards (1967).

Four liters of water from each sampler were filtered through glass-fiber filters (Gelman, type A),[6] stored in a dark desiccator at about 0° C, and returned to Seattle for chlorophyll *a* analyses. A layer of $MgCO_3$ was added to the filter prior to filtration. Chlorophyll *a* concentrations were determined by the method of Richards with Thompson (1952) but were computed with equations given by Parsons and Strickland (1963). Chlorophyll samples on the glass-fiber filters were ground in a tissue grinder as suggested by Yentsch and Menzel (1963). The resulting suspension was filtered through a very-fine-porosity (VF) fritted-glass disk under pressure, and the cake of residue remaining on the disk was stirred with a few ml of 90% acetone and refiltered. Absorbances at 750 mμ, which indicate turbidity, of the resulting effluents were seldom more than 0.010 per cm of light path and then only in the more highly colored samples. A series of 20 tests showed no more than traces of pigment remaining in the residue after the first wash. The above treatment for separating residue from samples was preferable to centrifugation because it resulted in generally lower turbidity and more complete recovery of extract.

Total incident solar and sky radiation (over the wavelength range 0.3 to 3 μ) was continuously measured and graphically recorded by a pyranometer. Although the photosynthetically active portion of the spectrum is roughly half the total radiation (Edmondson, 1956), total radiation values were used in productivity calculations. Salinity and temperature were measured and standard weather observations were recorded near the productivity stations (Larrance, 1971).

Measurements of productivity, chlorophyll *a*, and nutrients at various depths were integrated to the bottom of the euphotic zone (designated here as that depth where light intensity is 1% of the surface intensity), or other specified depth, and the integrated values expressed per square meter of sea surface. Mean values for several oceanographic domains were computed by weighting the values according to distances between stations. Details of the calculations were given in Larrance (1971).

PHYSICAL OCEANOGRAPHY

The physical oceanography of the Pacific Subarctic Region has been described by Fleming (1955), Dodimead, Favorite, and Hirano (1963), and Tully (1964). On the basis of data from Ocean Station "P" (lat 50° N, long 145° W), Dodimead et al. (1963) divided the upper 1,000 m of the Subarctic Pacific Region into three permanent zones: an upper zone from 0 to about 100 m depth; a halocline from about 100 to 200 m through which the salinity increases downward by about 1‰; and a lower zone from about 200 to 1,000 m. During the spring and summer, warming of the surface layers causes a temporary thermocline in the upper zone which is subsequently destroyed by cooling in the autumn. Consequently the lower limit of the wind-mixed upper layer ranges from about 30 to 60 m in the spring and summer and extends to the top of the permanent halocline at 100 m during winter. The upper zone in the Subarctic Pacific has been divided by Dodimead et al. (1963) into Coastal, Alaskan Stream, Central Subarctic, Western Subarctic, and Transitional Domains (Figure 2).

The Coastal Domain south of the Aleutian Islands lies over the continental shelf and is strongly influenced by Bering Sea water mixed

[6] Gelman Instrument Co., P.O. Box 1448, Ann Arbor, Mich. 48106.

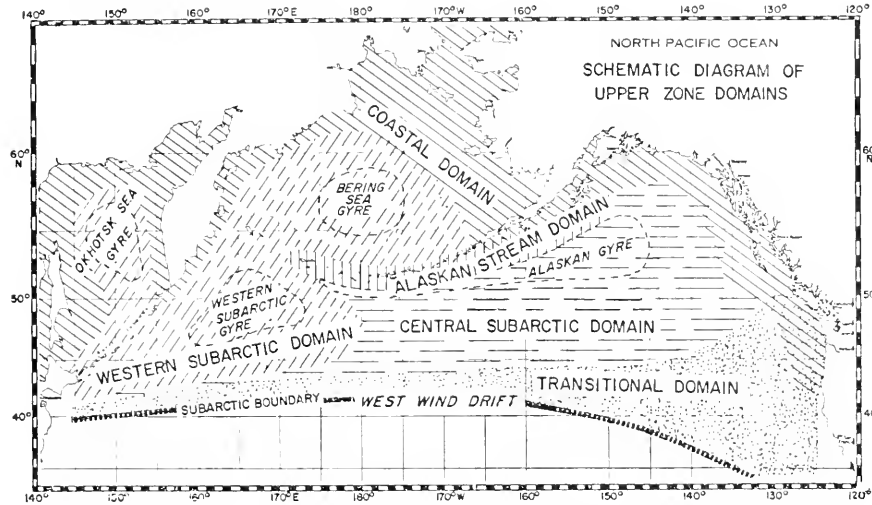


FIGURE 2.—Diagram of upper zone domains in the Subarctic Pacific Region (after Dodimead, Favorite, and Hirano, 1963).

through passes between the islands. To distinguish the Coastal from Alaskan Stream Domains, coastal water was arbitrarily defined by surface salinities greater than 32.9‰. The Alaskan Stream, described in detail by Favorite (1967), flows westward out of the Gulf of Alaska with velocities as high as 100 cm/sec. It is diluted by runoff from Alaska and can be detected by low salinity (less than 32.6‰) at the surface. The Central Subarctic Domain is an area of weak and variable currents bounded on the north by the Alaskan Stream and on the south by the Subarctic Current, which flows eastward at velocities between 5 and 20 cm/sec (McAlister et al., 1970). The Subarctic Current separates the Central Subarctic Domain from the Transitional Domain, which extends southward as far as the northern boundary of the Subtropical Region and is also an area of weak eastward flow. The nomenclature given by McAlister et al. (1970) was slightly different from that applied to the upper zone domains of Dodimead et al. (1963), but it was based in part on features below the upper zone. For purposes of the present paper, the definitions of the upper zone domains as given in Dodimead et al. (1963) were used; the Subarctic Current, which originates in the Western Subarctic Domain, was thus included in the Central Subarctic Domain.

The Transitional Domain has been further divided into two areas (T-1 and T-2) on the basis of the salinity in the upper 50 m. The division between areas T-1 and T-2 was set in August 1967 at lat 47° N, where surface salinity was a maximum and decreased to the north and to the south at least as far as lat 46° N. The Transitional Domain was also divided in September 1966, when the northern area (T-1) extended from lat 47°05′ N to a relatively sharp horizontal salinity gradient at lat 43°35′ N. The southern area (T-2) extended to lat 40°45′ N, where the boundary between transitional and subtropical waters was found. These divisions of the Transitional Domain are somewhat arbitrary but tend to be corroborated by biological and chemical characteristics.

PRIMARY PRODUCTIVITY ESTIMATES ADJUSTED FOR DIFFERENCES OF LIGHT INTENSITY

Because productivity was measured in natural light which differed (by as much as fivefold) in total insolation from day to day, productivity values were adjusted by two methods to permit comparison of productivity estimates under similar light conditions for purposes of detecting possible differences in productivity among oceanographic areas. One method applied the relation given by Ryther (1956) and Ryther and Yentsch (1957) of relative daily productivity beneath a unit of sea surface to total daily surface radiation. The measured daily light intensities were averaged for each cruise and the corresponding values of R (photosynthetic rate relative to photosynthesis at light saturation), defined by Ryther and Yentsch (1957), were determined for the cruise mean of daily light and for the light observed on the particular day in question (R_{av} and R_m, respectively). Adjusted productivity was then computed by $P_R = P_m \times R_{av}/R_m$ where P_R and P_m are the adjusted and observed productivities integrated through the euphotic zone and have the units of mg C m^2 per day. This procedure amounts to using the shape of Ryther's (1956) curve but not his absolute values for estimating productivity.

Since Ryther derived his curve from photosynthesis measurements of phytoplankton from Woods Hole Harbor, that relationship is likely to differ from similar curves based on measurements from other areas. An attempt was made, therefore, to establish a simple empirical relation to estimate productivity in the mid-Subarctic Pacific Region from chlorophyll and light data obtained during the *Kelez* cruises. The regression of measured daily productivity (P_m) per unit of chlorophyll a (C_a) in the euphotic zone

$$\left(\text{where } P_m/C_a = \frac{\text{mg C assimilated/m}^2 \text{ per day}}{\text{mg chlorophyll } a \text{ m}^2}\right)$$

on daily light intensity measured on the ship's deck was computed (Figure 3). Only data from those stations where a full day's productivity and light were measured were used for the relation.

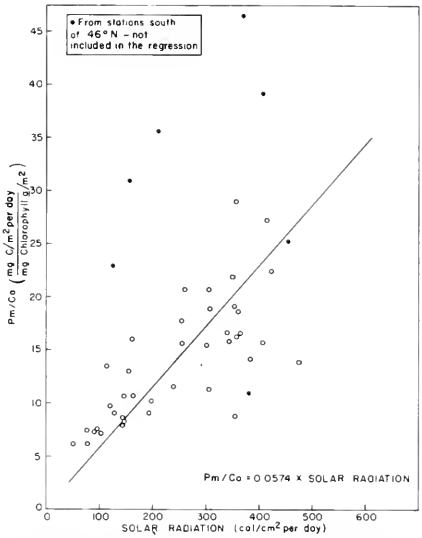


FIGURE 3.—Relation between the ratio of daily primary productivity to chlorophyll a (P_m/C_a) in the euphotic zone and daily solar radiation above the sea surface in the Subarctic Pacific Region, 1966-67.

The intercept of the regression and the axes was not significantly different from the origin. The variability was generally large, as might be expected from data of this kind, and was especially high for stations in the southern portion of the study area (transitional and subtropical water). This variability suggests that productivity responses to the environment in the Transitional Domain and subtropical water were probably different from those north of about lat 46° N. Furthermore, relatively few measurements were taken south of lat 46° N—not enough for seasonal comparisons. For these reasons, the regression was computed for only those stations north of lat 46° N.

Productivities under average daily light intensities for each cruise (P_K) were estimated by multiplying the chlorophyll a measured in the euphotic zone at a station by the estimate, from the regression, of P/C_a corresponding to the

average daily light intensity for the cruise. Although the relation was based on data taken north of lat 46° N, it was used to estimate productivity in June 1966 for stations as far south as lat 44° N (Figure 4).

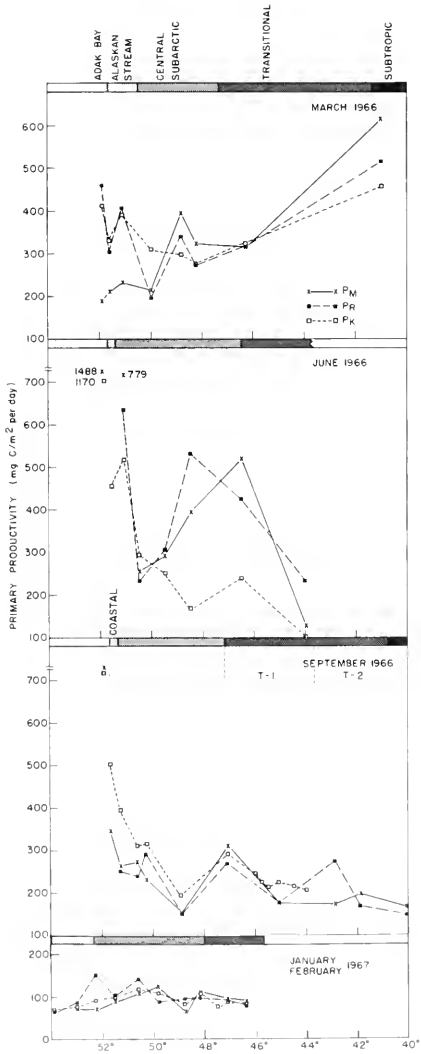
These methods give rough approximations at best but probably indicate productivity responses to seasonal-average light conditions more accurately than those given by measured productivity values affected by large day-to-day fluctuations of light. The means of P_R for each cruise were higher than means of P_R except in September (Table 2, Figure 5). The higher P_R values may mean that the photosynthetic efficiency of the subarctic Pacific phytoplankton is lower than the average efficiency of populations represented in Ryther and Yentsch's (1957) analysis. Other possible explanations might involve differences between our experimental procedures and Ryther's (1956).

AREAL AND SEASONAL DISTRIBUTIONS OF PRIMARY PRODUCTIVITY AND CHLOROPHYLL *A*

Differences in productivity and chlorophyll *a* among the upper zone domains do not appear to be consistent from season to season except that mean values in the Coastal Domain and Adak Bay were higher than those farther from shore (Figure 4, Table 2). The Coastal and Alaskan Stream Domains can be compared only in June-July 1967 because both areas were not sampled on any other single cruise. Productivity and chlorophyll *a* were substantially higher in coastal water than in the Alaskan Stream. Unlike other times of the year, productivity and chlorophyll values were similar in nearshore and offshore areas in March 1966 and January-February 1967, probably because low light intensities in these months limited production to about similar levels throughout the northern Subarctic Region. This effect was especially pronounced at Adak Bay, where productivity estimates in March were between 350 and 460 mg C m^2 per day but ranged between 840 and 2,400 mg C m^2 per day in late spring and through the summer. The lowest productivity during each cruise was in the Central Subarctic Domain except in winter, when productivity was uniformly low throughout the region. The mean productivity, however, was lower in the Central Subarctic Domain than in the other areas only in March 1966 and June 1967.

Daily carbon assimilation was normalized ($P\ C_a$) above to estimate productivity from chlorophyll and light measurements. The commonly used $P\ C_a$ ratio may also be considered as an index of the capacity of a population to photosynthesize under natural light and ambient nutrient and temperature conditions, and provides a basis for seasonal and areal comparisons. This ratio is similar to "turnover rate" (Cushing et al., 1958) and to the ratio discussed by Currie (1958), except that Currie used the concentration of the total complement of plant pigments instead of only chlorophyll *a*. Platt (1969) used an efficiency index (productivity/light energy) to normalize productivity measurements for comparison at designated chlorophyll concentrations by means of a regression of the efficiency index on chlorophyll. His method was similar to that used here except he could estimate productivity at individual depths. For analysis in this study, ratios of $P\ C_a$ were computed for measured productivities (P_m) and productivities adjusted for differences in light (P_R) by the curve of Ryther and Yentsch (1957).

Ratios of $P_R\ C_a$ were high at four of the nine stations at or south of lat 46° N (Figure 6). The southernmost stations in March and September 1966 were in subtropical water. In March, P_R (515 mg C m^2 per day) and P_R/C_a (39) were high, suggesting conditions of a phytoplankton bloom, whereas in September, P_R/C_a was lowest (10) of any observed during summer (Table 2). At lat 44° N in June 1966 and at lat 42°50′ N in September, $P_R\ C_a$ ratios were high (41 and 49), but P_R values were moderate (232 and 275 mg C m^2 per day) and chlorophyll values were unusually low (5.6 mg m^2 in each case). This combination of high P_R/C_a and low chlorophyll may be due to high carbon to chlorophyll ratios in the cells. At lat 46° N in August 1967, however, P_R and chlorophyll *a* were both relatively high (664 mg C m^2 per day and 16.8 mg/m^2); thus the high productivity and photosynthetic capacity ($P_R\ C_a$ = 40) of the popu-



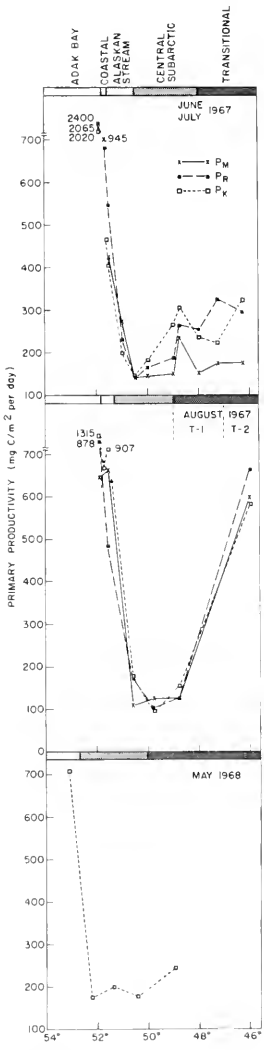


FIGURE 4.—Primary productivity in the mid-Subarctic Pacific Region, 1966-68.

TABLE 2.—Chlorophyll *a* in the euphotic zone, measured and estimated primary productivity values, and ratios of productivity to chlorophyll *a* in the mid-Subarctic Pacific Region (averaged within upper zone oceanographic domains) 1966-68.

Date and cruise no. and area	Chlorophyll *a* (mg/m³)	Primary productivity (mg C/m² per day)					
	C_a	P_m	P_R	P_K	P_m/C_a	P_R/C_a	P_K/C_a[1]
March 1966 (K1-66)							
Adak Bay	24.9	187	460	351	7.5	18.5	
Alaskan Stream	23.2	229	392	327	9.9	16.9	
Central Subarctic	17.8	305	265	251	17.1	14.9	
Transitional	19.6	317	317	276	16.2	16.2	
Subtropic	13.2	612	515	--	46.4	39.0	
46°-51°40′	19.3	292	303	272	15.1	15.7	14.1
June 1966 (P2-66)							
Adak Bay	64.3	1485	1492	1061	23.1	23.2	
Alaskan Stream	25.0	--	--	412	--	--	
Central Subarctic	14.5	422	429	239	29.1	29.6	
Transitional	9.4	324	328	155	34.5	34.9	
46°-51°40′	14.4	431	426	238	29.9	29.6	16.5
September 1966 (K3-66)							
Adak Bay	42.4	914	--	827	21.6	--	
Coastal	23.8	345	--	464	14.5	--	
Central Subarctic	11.9	195	199	232	16.4	16.7	
Transitional 1	11.7	222	197	228	19.0	16.8	
Transitional 2	6.9	187	217	--	27.1	31.4	
Subtropic	15.0	165	150	--	11.0	10.0	
46°-51°40′	12.8	204	201	250	15.9	15.7	19.5
January-February 1967 (K1-67)							
Alaskan Stream	9.1	71	80	60	7.8	8.8	
Central Subarctic	12.4	93	108	82	7.5	8.7	
Transitional	10.1	94	82	67	9.3	8.1	
46°-53°53′	11.2	88	96	74	7.9	8.6	6.6
June-July 1967 (K5-67 and K6-67)							
Adak Bay	99.4	2068	2396	1889	20.8	24.1	
Coastal	22.8	945	680	433	41.5	29.8	
Alaskan Stream	14.0	290	265	266	20.7	18.9	
Central Subarctic	11.1	165	193	211	14.9	17.4	
Transitional	13.2	171	299	251	13.0	22.6	
46°-51°40′	12.7	202	248	241	15.9	19.5	19.0
August 1967 (K7-67)							
Adak Bay	60.6	648	878	867	10.7	14.5	
Coastal	41.8	661	483	598	15.8	11.6	
Central Subarctic	6.3	118	138	90	18.7	21.9	
Transitional 1	7.2	127	127	103	17.6	17.7	
Transitional 2	16.8	598	664	240	35.6	39.5	
46°-51°40′	10.7	238	247	153	22.2	23.1	14.3
May 1968 (K2-68)							
Alaskan Stream	41.3			710			
Central Subarctic	10.6			182			
Transitional	14.3			246			
48°50′-53°	14.3			246			

[1] Mean values for each cruise.

lation were undoubtedly real. From these results, productivity and photosynthetic capacity south of lat 46° N appear to be neither characteristically high nor low, except in spring during bloom conditions, but fluctuate over a wide range.

Because relatively few measurements were made south of lat 46° N and the P_R/C_a ratios varied rather widely, only the data between lat 46° N and the Aleutian Islands were compared to determine seasonal patterns of production. If all the data are treated as if taken in the same calendar year, a seasonal pattern of productivity can be approximated (Figure 5). Productivity in winter was uniformly low throughout the area (P_R =96 and P_K = 74 mg C/m² per day). The mean chlorophyll *a* concentration (11.2 mg/m²), however, was similar to

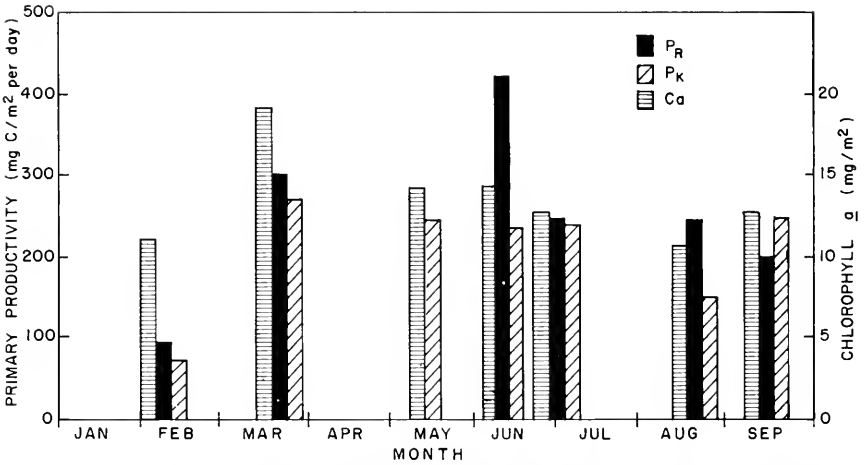


FIGURE 5.—Seasonal pattern of primary productivity and chlorophyll *a* in the mid-Subarctic Pacific Region between the Aleutian Islands and lat 46° N.

summer means. As might be expected, primary productivity was limited by low available light energy in the winter. The mean daily light intensity was low (116 cal/cm²), and the availability of light to the cells was further limited by their being distributed throughout the surface mixed layer, which reached well below the euphotic zone to the halocline at about 100 m.

In March, daily light intensities averaged 274 cal/cm² and thermal stratification had developed sufficiently to decrease the mixed-layer depth—thereby increasing exposure of the cells to light at shallower depths and consequently stimulating growth. The mean chlorophyll *a* concentration (19.3 mg/m²) was clearly higher than for any other season, and P_R and P_K (303 and 272 mg C/m² per day) were more than three times as high as in January and February (Figure 5, Table 2). The P_R/C_a and P_K/C_a ratios (16 and 14) were roughly twice those in midwinter, indicating that productivity was no longer limited by low light intensities. Although measurements in May 1968 were made at a considerable distance east of the Adak Line and may not be directly comparable, mean P_K values were remarkably similar in June 1966 and 1967 and May 1968 (238, 241, and 246 mg C/m² per day, respectively) as were the mean chlorophyll values (14.4, 12.7, and 14.3 mg/m², respectively).

In August, the mean P_K and P_R values differed significantly (P_K = 153 and P_R = 247 mg C/m² per day). A large part of this difference may be attributed to high productivity measured at lat 46° N in transition area T-2 (P_K = 240 and P_R = 664 mg C/m² per day). These estimates strongly influenced the means because the weights assigned to each in the averaging process were relatively large. The mean chlorophyll *a* concentration (10.6 mg/m²) was slightly less than in June, and the mean P_R/C_a (23) was higher than in June. In September, mean P_R and P_K were 201 and 250 mg C/m² per day, and the mean chlorophyll *a* was 13.0 mg/m². These values were somewhat similar to the other summer values, as were the ratios P_R/C_a (16) and P_K/C_a (20).

The general time-distributional pattern of productivity and chlorophyll *a* between lat 46° and 51°40′ N from January through September was drawn from the above results (Figure 5). Pro-

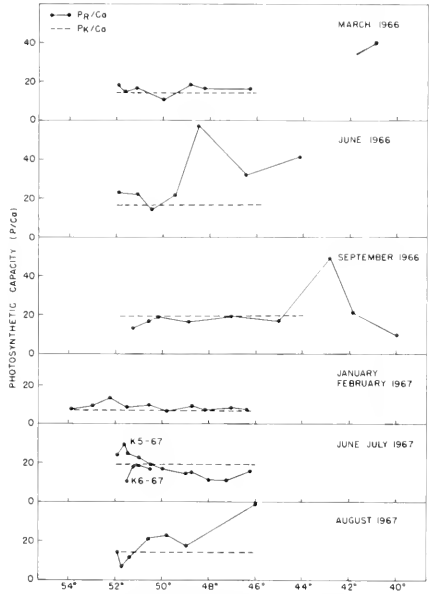


FIGURE 6.—Ratios of P_R/C_a and P_K/C_a in the Subarctic Region along long 175°-176° W, 1966-67.

duction was low in the winter, increased significantly in March, and was relatively steady at intermediate levels throughout the summer. Chlorophyll *a* also increased between February and late March as a consequence of high production but decreased significantly during the spring and decreased slightly throughout the summer. Some reasons for these changes can be inferred from the nutrient and zooplankton data and are discussed later.

Although primary production apparently continued at a high rate through the spring, chlorophyll *a* concentrations were significantly less in May and June than in March. Two probable reasons for this decrease are a decrease in cell chlorophyll content and an increased loss rate, primarily due to grazing. It is impossible to ascertain from the data which one of these causes was most important, but some trial calculations can help explore the problem. The standing stock (S_t) at t days (time between consecutive cruises) was predicted by the simple growth equation

$$S_t = S_0 e^{(a-b)t}$$

where S_0 is the initial standing stock and a and b are growth and loss coefficients. Standing stock was expressed in mg chlorophyll *a* /m², and P/C_a ratios were multiplied by 1 F (ratio of cell chlorophyll *a* to cell carbon) to give growth coefficients (a) in units of day^{-1}.

The growth rate (a) varied with time because P/C_a varied and $1/F$ was assigned values according to those reported elsewhere (Strickland, 1960; Eppley, 1968; and Strickland et al., 1969). Populations in nutrient-rich water under suboptimum light intensities, conditions extant in February and March, contain larger amounts of chlorophyll per unit carbon than those in nutrient-poor water under brighter light. Eppley (1968) found F values of about 30 for deep nutrient-rich water and 90 for shallow water depleted of nutrients. Values of $1/F$, therefore, were assumed to be 0.04 in February and March and 0.01 in June. The June value is probably too low because the water still contained ample nutrients (although lower than in March) for vigorous growth, but was selected to maximize the decrement afforded to decreasing cell chlorophyll content and therefore minimize S_t in June.

Results of the calculations show (Table 3) that changes in cell chlorophyll content could account for only part of the decrease in chlorophyll concentration between March and June. The loss coefficient (b) was computed for the period February to March and assumed to remain constant through June. The computed standing stock (S_t) in June was 1096, about 80 times as high as the observed value. (For comparison, standing stocks were also computed for $1/F = 0.04$ and $1/F = 0.01$, Table 3.) Since this (1096) is the minimum that could be expected from a loss of cell chlorophyll, grazing must have increased during the period to further decrease the chlorophyll concentration to its observed level. A concomitant increase in zooplankton corroborates this conclusion (discussed later).

TABLE 3.—Computed standing stock (S_t), expressed in units of chlorophyll, for various cell chlorophyll to carbon ratios ($1/F$) during spring 1966 in the mid-Subarctic Pacific Region.

Period	S_0	t	$1/F$	P/C_a	[1]$\bar{a}$	b	S_t
Feb.-Mar.	11.2	50	.04	7-15	0.433	[2]0.422	19.3
Mar.-June	19.3	80	.04-.01	15-20	0.472	0.422	[2]1096.
Mar.-June	19.3	80	.04	15-20	0.701	0.422	[2]9.3×10[10]
Mar.-June	19.3	80	.01	15-20	0.398	0.422	[2]28.0

[1] $\bar{a}$ is the mean growth coefficient calculated by assuming that P/Ca varied linearly with time as did $1/F$ in the second computation.

$$\bar{a} = a_0 + \frac{\triangle a}{t} \sum_{i=1}^{i=t-1} i$$

where $\triangle a = \triangle P/C_a\ [(1/F)_0 + 1/t \cdot \triangle 1/F] + (P/C_a)_0 \cdot \triangle 1/F$ and $\triangle$ is the difference between the initial and final values for the period.

[2] Computed values.

AREAL AND SEASONAL DISTRIBUTION OF NUTRIENTS

Nutrient concentrations were always higher in Adak Bay and the Coastal Domain than in the other areas and generally decreased toward the south (Table 4, Figure 7). Average concentrations of nutrients in the Central Subarctic water exceeded those in the Alaskan Stream only in June 1967. In winter of 1967, the nutrients were relatively high and varied little throughout the cruise area. Low nutrient concentrations at a few stations to the south in September correspond to lower P_R C_a ratios. The lowest average phosphate concentration in the upper 50 m, 10 mg-at/m^2, was in subtropical water. Nitrate-nitrite was undetectable in the upper 10 m at one station in area T-2 (lat 43° N) and totaled only 12 mg-at/m^2 in the upper 50 m, whereas the minimum silicate observed was 187 mg-at/m^2 at the same station. Apart from the low nutrient values measured south of about lat 44° N in September and possibly in subtropical water in March, nutrients appeared to be in sufficient abundance to support vigorous phytoplankton growth. Even for these areas of low concentrations, productivity did not appear to be severely limited, as evidenced by the P_R C_a ratios, but was probably somewhat suppressed.

To obtain a seasonal pattern of changes, the data on nutrients, like those on productivity, were treated as composite measurements from the same year. The changes of mean nutrient concentrations between lat 16° and 51°40′ N from season to season were not large. In mid-winter all nutrients were relatively abundant, as were phosphate and silicate in March. (Nitrate was not measured in March.) Phosphate decreased between March and June 1966 from 78 to 56 mg-at/m^2, the largest fractional change measured during this study for any of the nutrients. In June 1967, nitrate and phosphate concentrations were nearly equal to those in winter, but concentrations of silicate were lower. The apparent difference in the phosphate fluctuations between the 2 years could be the result of a shift in timing of the periods of high primary productivity, differences in supply by circulation, or an overall net difference in the balance between phosphate assimilation and supply for the year. By August phosphate and silicate had increased to quantities considerably higher than those in winter, but in September phosphate was lower and silicate was only slightly higher than in winter.

The low nutrient concentrations to the south in the summer lend support to the proposal by Anderson (1969) that a trans-Pacific band of chlorophyll occurs between the seasonal and permanent pycnoclines in the summer. His measurements indicate that the algae are produced in situ at these depths (50 to 75 m) which receive less than 1% of the light energy at the sea surface. This band lies between lat 35° and 45° N and is coincident with and could explain the occurrence of a layer of maximum oxygen content (Reid, 1962). Anderson also found that nitrate in the surface mixed layer above the chlorophyll band was nearly absent, having been used up in the spring during high primary production. A nitrate gradient through the deep

TABLE 4.—Dissolved nitrate, phosphate, and silicate in upper 50 m of mid-Subarctic Pacific Region (averaged within upper zone oceanographic domains), 1966-68.

Cruise no. and area	Nitrate $mg\text{-}at/m^2$	Phosphate $mg\text{-}at/m^2$	Silicate $mg\text{-}at/m^2$
K1-66			
Adak Bay	--	104.6	2,849
Alaskan Stream	--	88.8	1,990
Central Subarctic	--	81.2	1,903
Transitional	--	60.2	1,195
Subtropic	--	29.2	512
46°-51°40′	--	77.8	1,754
P2-66			
Adak Bay	--	94.9	2,871
Alaskan Stream	--	81.2	1,978
Central Subarctic	--	56.9	1,491
Transitional	--	49.6	1,155
46°-51°40′	--	56.0	1,480
K3-66			
Adak Bay	954	90.4	2,499
Coastal	--	88.7	2,331
Central Subarctic	583	75.4	1,692
Transitional I	492	56.7	1,143
Transitional II	64	16.8	280
Subtropical	76	10.0	335
46°-51°40′	579	72.0	1,597
K1-67			
Alaskan Stream	571	83.4	1,594
Central Subarctic	661	82.0	1,665
Transitional	599	69.0	1,314
46°-53°53′	627	79.0	1,561
K5-67			
Adak Bay	772	101.7	2,192
Coastal	1,160	117.4	2,806
Alaskan Stream	534	65.8	811
Central Subarctic	790	89.0	1,704
Transitional	248	42.2	588
46°-51°40′	579	78.6	1,247
K6-67			
Alaskan Stream	554	56.9	942
Central Subarctic	800	90.6	2,026
K7-67			
Adak Bay	1,111	113.6	3,012
Coastal	1,020	120.7	2,666
Central Subarctic	596	88.2	1,854
Transitional I	724	97.3	1,883
Transitional II	276	49.2	792
46°-51°40′	610	86.4	1,725
K2-68			
Alaskan Stream	--	60.0	868
Central Subarctic	--	63.1	1,022
Transitional	--	105.7	1,415

chlorophyll maximum suggested that nitrate diffused toward the surface from deeper water was completely assimilated in the deep layer. During the present study, chlorophyll maxima were found at 50 m in June 1966 at lat 44° N and at 100 m in September at lat 43° N. Nitrate was not measured in June but it was undetectable in

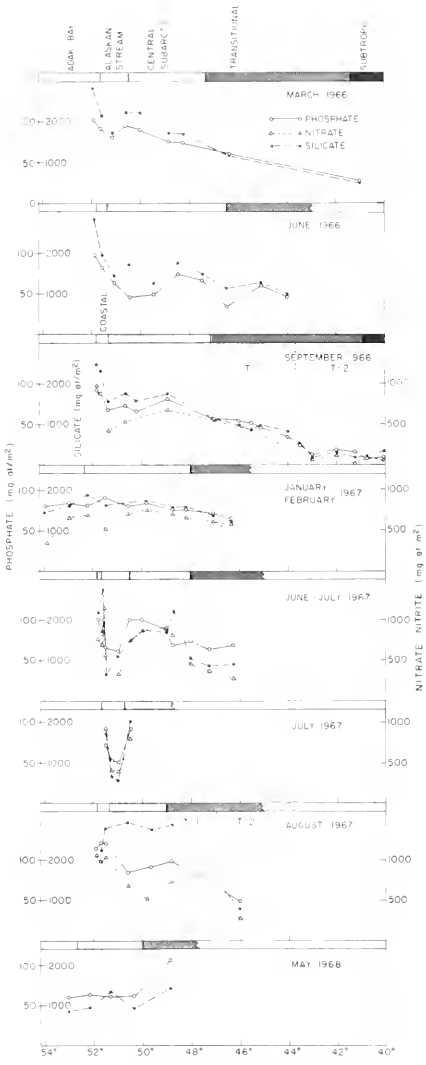


FIGURE 7.—Dissolved nitrate-nitrite, phosphate, and silicate in the upper 50 m of the mid-Subarctic Pacific Region, 1966-68.

the upper 10 m at lat 43° N in September. The deep chlorophyll layer at other stations could easily have been missed because sampling below the 1% light level was at standard depth.

PHOSPHATE CHANGES AND THEIR RELATION TO PRIMARY PRODUCTION

An attempt was made to draw qualitative inferences from phosphate data about the relative levels of primary production between cruises to obtain a somewhat more detailed picture of the seasonal productivity pattern. The major factors generally affecting changes in dissolved phosphate concentration in the oceans are: (1) utilization by primary producers (P_u), (2) regeneration by zooplankton and bacteria (P_r), and (3) advective changes (P_a). The relation among these factors is given by the formula:

$$P_0 = P_u + P_r + P_a,$$

where P_0 is the net change of phosphate concentration with time, P_u is negative, P_r is positive, and P_a can be of either sign. Estimates of P_u between cruises were calculated by applying a ratio of carbon to phosphorus (C:P) to the carbon-14 data as discussed below, P_a was estimated from calculations of vertical velocities and phosphate concentrations measured at depth, and P_0 was calculated from measurements of phosphate concentrations; however, no adequate estimate of P_r was possible. Two major assumptions were necessary to evaluate the above parameters:

1. The uptake ratio of C:P = 40 by weight (Strickland and Parsons, 1965). This value relates the C and P content of cells, but not the amounts assimilated. It is applied here, however, to carbon-assimilation rates measured by the carbon-14 method, which measures rates between net and gross production. The resulting estimate of phosphorus uptake, therefore, will be larger than the actual amount of phosphorus retained in new cell material.

2. Phosphate concentrations and their in situ changes were uniform within individual upper-zone domains. This assumption permits us to neglect the effect of horizontal advection.

Since nutrients were generally abundant in the Subarctic Region and changes were small during the year, circulation and regeneration must have supplied dissolved nutrients to the upper zone at rates sufficient to keep pace with their utilization, despite high assimilation rates by the algae during spring and summer. The amounts of phosphate supplied to the upper 50 m by upwelling were estimated from calculations of monthly mean vertical velocities (Wickett, 1966, 1968)[7] and observed phosphate concentrations at 50 m (Table 5). Wickett listed meridional components of Ekman and total transport for alternate points on a grid of 5-degree units of latitude and longitude. To obtain the phosphate estimates, the average of monthly mean vertical velocities for grid points at lat 45° N, long 175° W and at lat 50° N, long 180° W were used as single monthly estimates applicable to the Adak line of stations north of lat 45° N. To obtain the net amount of phosphate exchanged through the 50-m surface, the net vertical displacement of water during each month was multiplied by mean phosphate concentrations at 50 m. The computed vertical velocities refer to the bottom of the Ekman layer which extends to the halocline at 100 m in winter but is limited by the thermocline in summer to as shallow as 30 m. The error incurred, however, by applying the velocities to 50 m rather than any other level was probably within the range of precision of the estimated velocities.

Such estimates of vertical transport of phosphate must be considered minimal, because the turbulent flux of properties across a surface cannot be computed by using mean velocities. That is, mean vertical velocities indicate net upward flow, although water and its associated properties actually move up and down across horizontal surfaces. When phosphate concentration increases with depth (as it usually does), the shallower water loses less phosphate by downward flux than it gains by equivalent up-

[7] Wickett, W. P. 1966. Ekman transport computations for the North Pacific Ocean, 1966. Fish. Res. Board Can., Manuscr. Rep. Ser. (Oceanogr. Limnol.) 229, 92 p. (Processed.)
1968. Transport computations for the North Pacific Ocean, 1967. Fish. Res. Board Can., Tech. Rep. 53, 92 p. (Processed.)

TABLE 5.—Changes of dissolved phosphate in upper 50 m of mid-Subarctic Pacific Region (attributable to vertical transport and assimilation by phytoplankton) and their relation to measured concentrations (shown in parentheses).

Period	P_0 mg P/m² per day (mg P/m²)	P_a mg P/m² per day (mg P/m²)	P_u mg P/m² per day (mg P/m²)	Residual change of P[1] mg P/m² per day (mg P/m²)
1966				
March-early June	−8.1 (−680)	1.0 (80)	−6.4 to −9.3 (−530 to −760)	−2.7 to 0 (−230 to 0)
Late June-Sept.	5.4 (500)	1.5 (140)	−5.5 to −8.4 (−500 to −770)	9.4 to 12.3 (860 to 1130)
1967				
Feb.-June	0 (0)	2.7 (310)	−4.0 to −4.3 (−570 to −620)	1.3 to 1.6 (260 to 430)
July-Aug.	4.3 (240)	−1.7 (−100)	−4.9 to −6.2 (−280 to −350)	10.9 to 12.2 (620 to 690)

[1] See text for definition.

ward motion; the net upward flux of phosphate, therefore, is underestimated when mean vertical velocities are applied.

Values of P_u for the periods between cruises were estimated from productivity data. The lesser values of the two productivity estimates (P_K or P_R) from each of two succeeding cruises were averaged, as were the greater values. These averages represented the limits of the range of mean productivity during the period between cruises. For example, in the summer of 1966 (when in June, $P_K = 238$ and $P_R = 426$ mg C/m² per day, and in September, $P_K = 250$ and $P_R = 201$ mg C/m² per day), the range of mean productivity during the period was from 220 (the average of 238 and 201) to 338 (the average of 426 and 250) mg C/m² per day. The limits of the ranges were divided by the C:P ratio (40) to obtain the daily rate of phosphorus uptake in milligrams within a 1-m² cross-sectional column of the euphotic zone (Table 5). This rate was considered equivalent to the uptake in the upper 50 m. No error was incurred by this approximation when the euphotic zone was no deeper than 50 m. The "residual changes" of P were the changes unaccounted for by P_a and P_u; thus they included regeneration, other changes not evaluated, and measurement errors:

$$\text{residual change} = P_0 - (P_a + P_u).$$

Although the accuracy of these estimates was low, the direction that P_a and P_u are likely to be in error is known and the direction of error of the residual changes can be deduced. As the absolute values of P_a were minimal and those of P_u were too large (and P_a was either positive or negative and P_u was always negative), the sum $P_a + P_u$ tended to be underestimated. The residual changes, therefore, tended to be overestimated.

The residual changes during similar seasons in the 2 years indicate similar trends (Table 5). Negative values during spring 1966 show that phosphorus assimilation, and hence primary productivity, must have averaged more than that calculated, even if no regeneration occurred. If phosphate regeneration is assumed to be zero, the productivity during spring 1966 could have been as much as 40% higher than that calculated to account for the changes in measured phosphate. Although regeneration rates were probably lower than in summer, some regeneration probably occurred, and therefore the residual change would have been greater and the productivity even higher. Clearly, spring phytoplankton production in 1966 must have been substantially greater than the measured productivities.

Larger residual changes in the summer indicate higher phosphate regeneration rates than in spring. Phosphate turnover times ranging from about one to several months have been reported (Ketchum, 1962). According to Ketchum, excretion by zooplankton accounts for large portions of regenerated phosphate as well as inorganic nitrogenous compounds. The residual changes were correlated with zooplankton

biomass in the Subarctic Region, which was roughly three times higher in summer than in February or March (Donald S. Day, unpublished data).[5] If phosphate regeneration accounted for all the residual changes in summer, 45 to 50% of the phosphate in the water would be renewed by regeneration in 3 months. In contrast, the upwelled phosphate supplied only about 6% of the total concentration in summer of 1966 and phosphate was lost from the upper layers in 1967 by mean velocities downward. According to the computed values (Table 4), however, in the summer the residual change of phosphate was roughly twice that removed from the water by plants. If the residual change is assumed to be mostly due to regeneration, therefore, the zooplankton would have had to release twice as much phosphorus as was taken up by the algae during the same period. A more likely explanation is that more phosphate was supplied from below than is indicated by the P_a values and the consequent residual changes would be less. In either case, in situ regeneration by zooplankton appears to be a major source of nutrients supplied throughout the summer in the mid-Subarctic Pacific Region.

At Ocean Station "P" primary productivity accounted for the entire loss of phosphate between March and August (Parsons, 1965),[6] suggesting that regeneration was negligible. But, since zooplankton at Station "P" was sufficiently abundant to graze the phytoplankton to a stable level (McAllister, Parsons, and Strickland, 1960), it would seem that some phosphate regeneration should have occurred.

RELATION OF ZOOPLANKTON BIOMASS TO CHLOROPHYLL AND PRIMARY PRODUCTION

Data on zooplankton abundance and chlorophyll were compared to determine if these two variables were correlated. The smaller zooplankters were sampled by raising a ½-m NORPAC net (mesh opening 0.33 mm) vertically from 150-m depth to the surface at about 1 m/sec. Displacement volumes of the catches weighted for distance between stations were averaged between lat 46° N and 51°40′ N for each cruise. The mean volumes in February and March were about 0.070 ml/m³ of water strained and ranged from about 0.250 to 0.280 ml/m³ in summer, except in August 1967 when the mean volume was 0.550 ml/m³ (Day, see footnote 8). Thus, the zooplankton standing stock increased to about four times its winter level sometime after the phytoplankton increase in March. Grazing by the zooplankton apparently occurred early enough to crop down the algae, thereby limiting primary productivity before it reached sufficiently high levels to deplete the nutrients from the upper layers. A relatively steady state of grazing pressure and phytoplankton standing stock seemed to hold during the summer (at least in summer 1966). These findings agree with the conclusion of McAllister et al. (1960) that zooplankton grazing limited primary production at Ocean Station "P" by maintaining the phytoplankton standing stock at relatively low concentrations.

The relation between zooplankton displacement volumes and chlorophyll *a* concentrations (Figure 8) shows a negative correlation, further corroborating the above conclusion. The regression of chlorophyll *a* on zooplankton includes only those stations north of lat 46° N, except in coastal water and Adak Bay, and excludes all winter data. As shown previously the nearshore and transition waters exhibit chemical and biological features, which indicate ecological areas somewhat distinct from the area between. The winter data were also excluded from the regression because productivity was limited by insufficient light. Chlorophyll *a* concentrations south of lat 46° N showed no apparent relation to the amount of zooplankton present. Chlorophyll in Adak Bay and coastal waters was always significantly higher than estimated from the regression, except in March in Adak Bay. All of the high chlorophyll values were near shore and associated with intermediate quantities of zooplankton.

[5] Donald S. Day, Oceanographer, Natl. Mar. Fish. Serv. Biol. Lab., Seattle, Wash.

[6] Parsons, T. R. 1965. A general description of some factors governing primary production in the Strait of Georgia, Hecate Strait and Queen Charlotte Sound, and the N.E. Pacific Ocean. Fish. Res. Board Can., Manuscr. Rep. Ser. (Oceanogr. Limnol.) 193, 34 p. (Processed.)

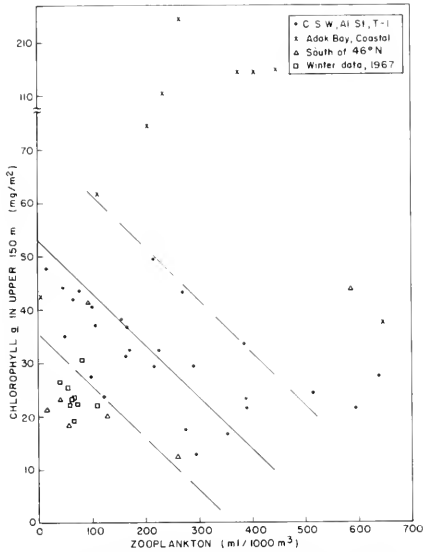


FIGURE 8.—Relation of chlorophyll *a* to net zooplankton in the upper 150 m of the Subarctic Pacific Region, 1966-68. Dashed lines represent 95% confidence limits.

The zooplankton value at the only nearshore station (Adak Bay) in March was the lowest observed. These data suggest that the zooplankton increase occurred later in the spring in coastal areas than farther offshore, a condition which could have permitted the phytoplankton bloom to reach much higher levels near shore before being controlled by grazing.

ANNUAL PRIMARY PRODUCTION

The annual primary production between lat 46° and 51°40′ N was estimated from values of P_K, P_R, and P_M. The data from all cruises were combined into a single composite year for apportioning productivity to time periods. The lowest estimate was for P_K (72 g C/m² per year); P_m and P_R were somewhat higher (82 and 85 g C/m² per year). If the estimate of primary productivity from phosphate changes is correct, annual productivity could be as high as 100 g C/m². These estimates are considerably higher than those reported by McAllister (1969), moderately higher than those of Koblents-Mishke (1965), and lower than those of Anderson (in press).

No reason is apparent from the quantitative data for the difference between annual productivity at station "P" (48 g C/m² per day; McAllister, 1969) and that in the mid-Subarctic Region. If zooplankton is the main factor limiting productivity, more zooplankton and less chlorophyll should be expected at station "P" than south of the Aleutians; however, zooplankton density was lower and chlorophyll *a* concentrations were about the same or slightly higher at station "P." More detailed seasonal observations from the mid-Subarctic, as well as a comparison of plankton communities in the two locations, will probably be necessary to explain the observed differences in annual productivity.

CONCLUSION

The main conclusions drawn from the primary productivity and related data are listed below:

1. No significant differences in primary productivity were found consistently among the various oceanographic domains or other waters of the Subarctic Pacific Region except that Adak Bay and the Coastal Domain south of Adak Island were generally more productive than areas farther south.

2. The annual cycle of productivity is typical for temperate oceans: productivity is low in winter, increases in the spring until more or less of a bloom develops, and declines by summer to relatively steady intermediate levels. Productivity in autumn and early winter was not studied.

3. Light limits productivity during winter; less light penetrates the surface than during other seasons, and the greater thickness of the mixed layer in the upper zone further reduces the light available to the algae.

4. During spring and summer, nutrients and light are plentiful, but zooplankton appears to graze the standing stock of phytoplankton to relatively low levels—thereby limiting productivity of phytoplankton.

5. The main mechanisms of dissolved-phosphate supply in the upper 50 m of water during summer are in situ regeneration by zooplankton and upwelling. Regeneration supplies significant amounts of phosphate (perhaps more than is provided by upwelling) in the summer, but increased turbulence and upwelling in winter maintain high levels of nutrients throughout the upper 100 m of water.

6. The seasonal cycle and factors limiting productivity are similar to those at station "P" (lat 50° N, long 145° W), but some significant differences exist. Productivity in the study area is nearly twice that at station "P," and zooplankton biomass is also much greater. The change in measured phosphate concentrations at station "P" over the productive season can be accounted for by phosphate utilized by the algae and by phosphate supplied by upwelling. South of the Aleutian Islands, however, phosphate regenerated in situ must be invoked to balance the phosphate budget.

7. Annual primary production in the area studied is about 80 to 100 g C m^2.

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Iago, A NEW GENUS OF CARCHARHINID SHARKS, WITH A REDESCRIPTION OF *I. omanensis*

LEONARD J. V. COMPAGNO[1] AND STEWART SPRINGER[2]

ABSTRACT

A new genus, *Iago*, is proposed for *Eugaleus omanensis* Norman, 1939. *I. omanensis*, originally described from a single specimen, is redescribed from 16 additional specimens from the northern Arabian Sea continental shelf and slope between the Gulf of Oman and the Gulf of Kutch. Its presence in areas of low oxygen and the possibility of its occurrence in deeper waters of the Red Sea are discussed.

Norman (1939) described *Eugaleus omanensis* from a 280-mm female specimen, taken at 210-m depth in the Gulf of Oman. He placed it in *Eugaleus* Gill, 1864 (= *Galeorhinus* Blainville, 1816) with reservations because *omanensis* differed from all other species of *Eugaleus* in dentition and absence of a pronounced ventral caudal lobe. Norman noted that *omanensis* did not fit *Hemigaleus* Bleeker, 1852 because of dentition differences and lack of precaudal pits but he declined to establish a new genus for it.

Fowler (1941) overlooked *Eugaleus omanensis* in his review of Indo-Pacific elasmobranchs but later (1956) gave a description of the species condensed from Norman's account and allocated it to the genus *Galeorhinus*. Misra (1949) had earlier placed it in the same genus but this was not mentioned by Fowler.

Smith (1957) revised *Galeorhinus* but also overlooked *G. omanensis*. Compagno (1970) reviewed the systematics of *Hemitriakis*, *Galeorhinus*, and related genera. He considered *G. omanensis* generically distinct from *Galeorhinus* but did not propose a new genus in deference to this paper.

During the International Indian Ocean Expedition (IIOE) in 1963, the RV *Anton Bruun* on Cruise 4B conducted 109 trawling stations in transects along the continental shelf of the Arabian Sea between Bombay and the Gulf of Oman at depths from 15 to 375 m (Woods Hole Oceanographic Institution, 1965). Sixteen specimens of a small carcharhinid were included in these collections and sent to us through the Smithsonian Oceanographic Sorting Center. They were tentatively identified by us as *Galeorhinus omanensis* (Norman).

Marshall and Bourne (1964, 1967), in photographic surveys of benthic fishes, collected 30 photographs of a small carcarhinoid shark (about 2 ft long) at depths between 1115 and 2195 m in the Red Sea. They noted that their shark might be either a triakid or a carcharhinid but was not identifiable to genus or species. Comparison of Marshall and Bourne's photographs and sketch of their "mystery shark" with our specimens and Norman's account of *G. omanensis* led us to suspect that the "mystery shark" might be *omanensis*.

We then sent two specimens of the IIOE series to Dr. N. B. Marshall at the British Museum (Natural History). At our request he compared them with the holotype, the hitherto only known specimen of *Galeorhinus omanensis*, and confirmed our identification of the IIOE specimens. He also agreed that the IIOE *omanensis* are very similar to the Red Sea "mystery shark" of the photographs, but noted that final identification of the Red Sea species must await capture of specimens.

Differences between "*Galeorhinus*" *omanensis* and members of *Galeorhinus*, *Hypogaleus*, *Hemitriakis*, and all other carcharhinid genera warrant the erection of a new genus for "*Galeorhinus*" *omanensis*.

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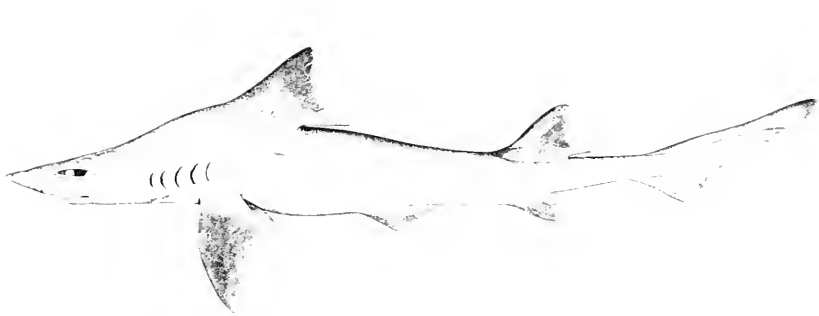

FIGURE 1.—*Iago omanensis*, from a 565-mm female deposited in U.S. National Museum. Drawing by Mildred H. Carrington.

Iago GENUS NOVUM

Eugaleus omanensis NORMAN, 1939, TYPE-SPECIES

Etymology

This shark, a namesake of the villain of Shakespeare's *Othello*, is a troublemaker for systematists and hence a kind of villain.

Diagnosis (Terminology Follows Compagno, 1970)

Iago (Figure 1) differs from most carcharhinoids in the extremely anterior origin of its first dorsal fin. Only *Isogomphodon oxyrhynchus*, a few species of *Carcharinus*, and the sphyrnid *Eusphyra blochii* rival *Iago* in this respect.

Iago is morphologically intermediate between the families Triakidae and Carcharhinidae as defined by Bigelow and Schroeder (1948) and Garrick and Schultz (1963). The characters of *Iago* strengthen the evidence presented by Compagno (1970) against separation of these families on simple nictitating lower eyelid and dental characters advocated by these writers. We follow Compagno in uniting, at least provisionally, the two families. *Iago* thus falls into the family Carcharhinidae (sensu lato).

Iago is far from the advanced and intermediate carcharhinid genera discussed by Compagno (1970). These genera include *Hemigaleus*, *Hemipristis*, *Galeocerdo*, *Scoliodon*, *Rhizoprionodon*, *Loxodon*, *Negaprion*, *Triaenodon*, *Lamiopsis*, *Isogomphodon*, *Carcharhinus*, *Hypoprion*, and *Aprionodon*. *Iago* differs from all of these in having a transitional, not internal, nictitating lower eyelid with edge nearly horizontal; shallow subocular pouch; teeth with strong basal ledges and grooves; teeth at symphysis only slightly smaller than adjacent ones; no precaudal pits; pectoral fin skeleton projecting less than halfway into fin; distal pectoral radials only as long as proximals, with parallel edges and truncate tips (not tapered and acute); caudal fin without projecting ventral lobe and lateral undulations of its dorsal margin in adults; cranium with a complete supraorbital crest (absent in advanced forms); and a spiral, not scroll, intestinal valve (*Hemigaleus* and *Hemipristis* are exceptional in also having spiral valves).

Iago differs from *Galeorhinus* and *Hypogaleus* as delimited by Compagno (1970) in having a transitional rather than internal nictitating low-

er eyelid in adults, with nearly horizontal edge; anterior nasal flap not greatly reduced; teeth without postlateral cusplets; an interdorsal ridge present; lateral trunk denticles much longer than wide in adults (about as long as wide in *Hypogaleus* and *Galeorhinus*); and no ventral lobe on caudals of adults.

From the curious *Leptocharias*, *Iago* differs in having a transitional rather than internal nictitating lower eyelid in adults; much larger spiracles; no nasal barbel; very weak gynandric heterodonty; teeth with primary cusps oblique, not erect, and lacking cusplets; sharp-edged, bladelike cutting teeth; fewer total vertebrae, 130 to 147 (198 to 214 in *Leptocharias*); spiral intestinal valve with about 5 turns (14 to 16 in *Leptocharias*); and an entire supraorbital crest (reduced in *Leptocharias* to isolated preorbital and postorbital processes).

Iago can be distinguished from *Hemitriakis*, *Furgaleus*, *Scylliogaleus*, the *Triakis-Mustelus* complex, *Proscyllium*, and *Eridacnis* by its more lateral eyes, in dorsal view nearly touching head rim, and its sharp-edged, monocuspidate teeth. In addition, *Iago* differs from *Hemitriakis* in having a transitional rather than external nictitating lower eyelid, noncarinate posterior teeth, more tooth rows (only 18 to 36 29 to 34 in *Hemitriakis*), weak transverse notches on teeth, and no ventral caudal lobe. *Iago* lacks the short, thick, rounded snout, nasoral grooves, and molariform teeth of *Scylliogaleus* and also has fewer tooth rows and series of teeth functional. Unlike *Furgaleus*, *Iago* lacks nasal barbels, erect cusps on its lower anterolateral teeth, and a ventral caudal lobe; also, *Iago* (Figure 2) has the nostrils definitely closer to the mouth than the snout tip (about equidistant in *Furgaleus*). *Iago* differs from most members of the *Triakis-Mustelus* complex in having fewer tooth rows and series of teeth functional; however, *Triakis semifasciata* rivals *Iago* in these respects. *Iago* does not have a pavement of molariform teeth as in *Mustelus*; also, its pelvic anterior margins are less than half the length of pectoral anterior margins (over half as long in *Triakis-Mustelus*). Finally *Iago* contrasts with *Proscyllium* and *Eridacnis* by its transitional, not rudimentary, nictitating lower eyelid; monocuspidate posterior teeth (not comblike); dorsal fin base midpoint closer to pectoral base termination than pelvic origins (vice-versa in *Proscyllium* and *Eridacnis*); second dorsal origin anterior (not over or posterior) to anal fin origin; intermedialia of vertebral centra strong wedges (not wedgelike in *Proscyllium* and *Eridacnis*); large papillae absent from gill arches and buccal cavity; and nostrils farther apart. *Iago* also lacks the clasper hooks, scyliorhinoid color pattern, and apparently the oviparous reproduction of *Proscyllium*.

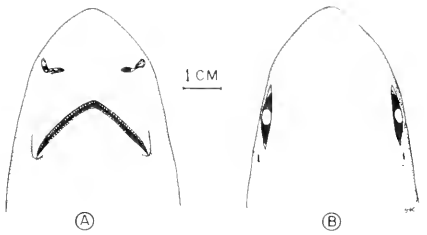

FIGURE 2.—*Iago omanensis*. A. Ventral side of head. B. Dorsal side of head.

GENERIC DESCRIPTION

Head flattened, its length from snout tip to fifth gill opening about $^1/_4$ total length.

Eye openings dorso-lateral, not visible in ventral view of head, openings elongate, about twice as long as high, with a well-developed posterior notch; nictitating lower eyelid transitional (Figure 3B), its edge nearly horizontal; secondary lower eyelid strongly differentiated, its edge thin; subocular pouch shallow, its lateral surface bare of denticles.

Slitlike spiracles, length about $^1/_7$ eye length, located about $^2/_7$ eye length behind and slightly below posterior eye notch; external gill slits moderately short, lengths in adults nearly equal, the longest $^1/_2$ to $^2/_3$ eye length; nostrils located about one-half as far from mouth as from snout tip, well separated, without nasoral grooves, widths about $1^2/_3$ to 2 times internarial distance, anterior nasal flap a short truncate lobe.

Mouth opening subtriangular, broad, 2 to 2½ times as wide as long; labial furrows extending around mouth corners, the upper furrows longer, extending anteriorly only to below eye pupils; large papillae absent from buccal cavity.

Teeth small (Figures 3A and 4), largest with greatest width at root about 1.5 mm in 457-mm female; tooth rows 46 to 55 37 to 45; 2 to 3 series functional along edges of jaws; teeth in mixed alternate and imbricate overlap pattern of Strasburg (1963); no serrations; premedial edge of crown in anteroposteriors convex, postlateral edge deeply notched forming a low postlateral blade on crown foot; all teeth with a strong basal ledge and groove, transverse ridges on crown foot; roots low, deep, with transverse

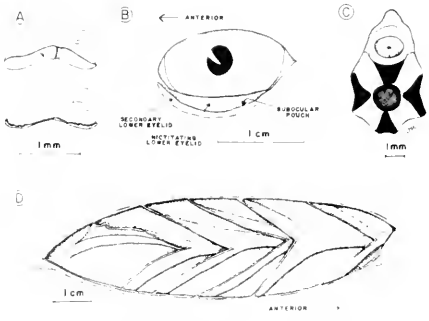

FIGURE 3.—*Iago omanensis*. A. Typical teeth: upper, buccal surface; lower, labial surface. B. Eye. C. Cross section of trunk vertebra; thoroughly calcified areas shown in black. D. Valvular intestine, one side cut away. Drawing by L. J. V. Compagno.

groove on attachment surface but transverse notch weak; teeth not noticeably protruding when mouth is closed.

Dignathic heterodonty very weak, with upper anteroposteriors having slightly higher crowns than lower ones; disjunct monognathic heterodonty indicated by differentiation of medials in one row on upper jaw and about 3 in lower; medials smaller, with erect primary cusps, large premedial and postlateral blades, and no cusplets; larger anteroposteriors are sharp-edged, compressed, bladelike cutting teeth with an oblique

FIGURE 4.—*Iago omanensis*. Teeth of right side of upper and lower jaws; labial aspect. Dotted lines indicate jaw symphysis.

primary cusp and no cusplets; anteroposteriors show moderate gradient monognathic heterodonty, with teeth becoming smaller, more oblique-cusped, and lower-crowned towards ends of dental band; posteriormost teeth with strong primary cusps; ontogenic heterodonty not known at present; gynandric heterodonty indicated only by slightly more erect cusp tips on anteroposteriors of adult males.

Trunk not markedly compressed, less than twice as high as wide, subtriangular in cross section; a low interdorsal ridge present; lateral dermal keels and precaudal pits absent from caudal peduncle.

Dermal denticles of trunk below first dorsal longer than wide, crown with a high, narrow ridge extending to tip of posteriorly directed cusp; a pair of lateral ridges weakly developed or absent, lateral cusps weak or absent.

Pectoral fins larger than first dorsal fin in area, their anterior margins about 1½ times as long as combined base and inner margin lengths; distal tip of adpressed pectoral about over its free rear tip when pectoral inner margin is held parallel to body axis; origin of pectoral below or slightly in advance of fourth gill opening; pectoral skeleton projecting less than halfway into fin, its longest distal radials about equal in length to corresponding proximal ones; distal radials with truncate tips and parallel edges.

Pelvic anterior margins less than half length of pectoral anterior margins; pelvic bases equidistant between first and second dorsal bases.

Claspers with pseudoperae, pseudosiphons, cover rhipidia, true rhipidia, and exorhipidia (Figures 5B and 5C); siphon sacs large, extending anteriorly to level of pectoral free rear tips (Figure 5A); margins of clasper cartilage rolled, with margins overlapping to form a tube; clasper hooks absent.

Origin of first dorsal fin far forward, varying in position from above fourth gill opening to slightly before pectoral axilla; midpoint of first dorsal base much closer to pectoral axilla than to pelvic origins; free rear tip of first dorsal anterior to pelvic fin origins.

Second dorsal nearly as large as first, its height about 70% of first dorsal height; its posterior margin strongly concave.

Anal smaller than second dorsal, slightly more than half its height, its base about ⅔ of second dorsal base length; its posterior margin nearly straight or shallowly concave; its origin posterior to second dorsal origin by about ⅕ to ½ of second dorsal base length; posterior ends of second dorsal and anal bases opposite.

Caudal without projecting ventral lobe-tip in adults, preventral margin slightly more than ⅓ of dorsal margin length; subterminal margin long, over half length of terminal margin; caudal dorsal margin length about ⅕ of total length; terminal sector of caudal about ⅓ of dorsal margin length; vertebral axis of caudal only slightly raised above body axis.

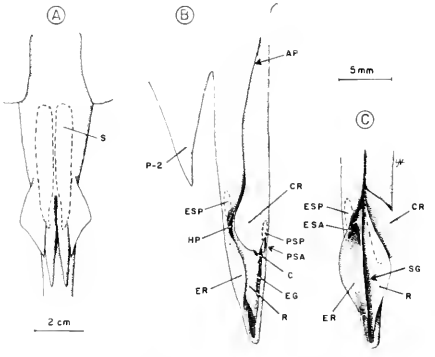

FIGURE 5.—*Iago omanensis*. A. Ventral aspect of trunk of mature male to show size and position of clasper siphons. B. Dorsal surface of left clasper. C. Partially expanded tip of left clasper. AP, apopyle; C, connection between rhipidion and cover rhipidion; CR, cover rhipidion; EG, epirhipidial groove; ER, exorhipidion; HP, hypopyle; P-2, pelvic fin; ESA, pseudoperal aperture; ESP, pseudopera; PSP, pseudosiphon pouch; R, rhipidion; S, siphon sac; SG, subrhipidial groove.

Vertebrae moderately numerous, 129 to 147 in total count ($N = 16$). Monospondylous precaudal (MP) centra 24.5 to 27.6% of total count; diplospondylous precaudal (DP) centra 33.6 to 36.1; and diplospondylous caudal (DC) centra 37.2 to 40.1 ($N = 8$). A ratios 120 to 162, B ratios 102 to 137 ($N = 11$). DP and DC centra more numerous than MP centra and nearly equal to each other, DP/MP ratio 1.22 to 1.46 and DC/MP ratio 1.38 to 1.63 ($N = 8$). Transition between MP and DP centra easily delimited on radiographs, over pelvic region. Posteriormost MP centra not greatly hypertrophied. DP centra of relatively uniform length throughout, not forming a stutter zone of alternating long and short centra.

Vertebral calcification pattern a modified version of White's (1937) "Maltese cross" pattern, without diagonal calcified lamellae; notochordal canal unusually large (Figure 3C); wedgelike intermedialia strongly developed.

Supraorbital crest of cranium strongly developed and entire.

Intestinal valve of spiral type, with about five turns.

Iago is apparently livebearing (see section in Reproduction below), but whether or not a yolk-sac placenta is formed cannot be determined from available specimens.

Iago omanensis (NORMAN, 1939)

Eugaleus omanensis Norman, 1939, p. 11, Fig. 3 (type-locality, Gulf of Oman); Compagno, 1970 (generic systematics).

Galeorhinus omanensis Misra, 1949, p. 21 (in list of Indian elasmobranchs, name only); Fowler, 1956, p. 17 (description, after Norman); 1967, p. 363 (in list of fishes of the world, name only).

MATERIAL

Seven males, 224 to 365 mm; nine females, 358 to 582 mm (Table 1); holotype, British Museum (Natural History) Reg. No. 1939.5.24.9, a 280-mm female from Gulf of Oman. Speci-

TABLE 1.—Oceanographic data for *Iago omanensis* from Final Cruise Report, *Anton Bruun* Cruise 4B, Woods Hole Oceanographic Institution (1965).

Length of specimen	Sex	IIOE station number	Date	Lat N	Long E	Bottom temperature	O_2 at bottom	Depth
mm						° *C*	*ml/liter*	*m*
312	♂	227A	11/19/63	22°38′	67°11′	22.39	0.77	110
224	♂	231A	11/20/63	23°13′	66°40′	19.89	0.24	183-155
457	♀	255A	11/30/63	25°50′	57°07′	24.47	2.40	92-95
427	♀	255A	11/30/63	25°50′	57°07′	24.47	2.40	92-95
440	♀	264A	12/02/63	25°02′	56°52′	20.02	0.60	291-272
480	♀	264A	12/02/63	25°02′	56°52′	20.02	0.60	291-272
472	♀	264A	12/02/63	25°02′	56°52′	20.02	0.60	291-272
363	♂	264A	12/02/63	25°02′	56°52′	20.02	0.60	291-272
358	♀	268A	12/03/63	24°12′	57°26′	16.24	0.28	364-368
565	♀	268A	12/03/63	24°12′	57°26′	16.24	0.28	364-368
582	♀	268A	12/03/63	24°12′	57°26′	16.24	0.28	364-368
335	♂	268A	12/03/63	24°12′	57°26′	16.24	0.28	364-368
365	♂	268A	12/03/63	24°12′	57°26′	16.24	0.28	364-368
395	♀	279B	12/09/63	24°13′	65°52′	19.34	0.22	170-192
304	♂	279B	12/09/63	24°13′	65°52′	19.34	0.22	170-192
295	♂	279B	12/09/63	24°13′	65°52′	19.34	0.22	170-192

mens are deposited in the collections of the U.S. National Museum, California Academy of Sciences, and the British Museum (Natural History).

DESCRIPTION

Proportional dimensions as percentages of total length are given for our 16 specimens in Table 2.

In lateral view head outline slightly convex dorsally and ventrally, its outline tapering smoothly to snout tip; outline of head in dorsal view parabolic in shape, the sides converging at a narrow angle from gill openings to nostrils but then converging at a much wider angle to snout tip; head broad, width at spiracles about half its length; interocular slightly less than width of head at eyes; head subquadrate in transverse section at eye pupils.

Snout tip narrowly rounded in dorsal view, bluntly pointed in lateral view; preoral length 1.1 to 1.4 in mouth width.

Eye length equal to or slightly longer than distance between nasal apertures; eye openings subelliptical, rounded anteriorly.

Nictitating lower eyelid (NLE) a variety of Compagno's (1970) transitional type, intermediate in form between "nictitating folds" and "nictitating membranes" of authors (Figure 2B); its anterior edge smoothly confluent with edge of upper eyelid; secondary lower eyelid (SLE) originating beneath origin of NLE; posteriorly NLE merges with SLE and continues as single edge to notch; subocular pouch or pocket between NLE and SLE extends beneath anterior two-thirds of eye; SLE edge sharp and thin with NLE is artificially raised to the limits of its travel; dermal denticles present on outer face of NLE. The structure and the kinetics of the NLE in *Iago omanensis* suggest that it cannot cover the potserior third of the eye when raised as it can in adults of *Galeorhinus* and higher carcharhinoids.

Spiracles open below level of posterior eye notch and are posterior to eye by distance about equal to their own length.

Gill openings increasing in size from first to third by small increments, with fourth slightly shorter than third and fifth about half length of fourth, becoming more dorsally situated from anterior to posterior, with upper origin of first opposite midpoint of fifth. Definite gill rakers not developed, but small dermal mounds are present on gill arches.

Nostrils relatively large, their openings obliquely directed anterolaterad; anterior nasal flap varying from subtriangular to lobate; a small inner nasal flap present dorsal to anterior nasal flap and concealed by it; posterior nasal margin with an elongated process (stiffened by ala nasalis) that projects obliquely dorsomedially into nasal cavity.

TABLE 2.—Proportional measurements of *Iago omanensis* expressed as percentages of total lengths; measurement method follows Bigelow and Schroeder (1948).

	Male 335 mm	Range in 7 males	Female 565 mm	Range in 9 females
Tip of snout to:				
Front of mouth	7.2	6.1-7.6	5.8	5.7-6.4
First gill opening	19.1	17.4-20.0	16.8	16.2-17.8
Last gill opening	24.2	24.2-27.5	23.4	23.0-24.5
Origin pectoral fin	23.3	23.3-25.9	22.7	21.2-23.5
Origin first dorsal fin	25.9	25.3-29.5	24.8	24.0-27.1
Origin pelvic fins	42.8	41.6-45.9	43.9	42.7-46.5
Origin second dorsal fin	56.8	56.8-60.6	58.4	57.8-59.9
Origin anal fin	58.3	58.3-63.4	59.2	59.2-63.3
Anus	44.7	43.5-49.1	45.1	44.5-47.4
Distance between fin base:				
First and second dorsals	23.5	21.4-25.3	24.2	21.9-24.6
Pectoral and pelvic	16.1	13.7-16.1	18.6	16.8-20.0
Pelvic and anal	11.0	11.0-12.3	11.5	11.0-13.2
Anal and lower caudal	8.9	7.2-10.5	8.5	7.5-9.7
Length eye opening	5.1	4.2-5.1	4.1	3.3-4.1
Least internasal distance	4.0	3.6-4.2	3.7	3.3-3.9
Width mouth	7.2	7.7-9.4	7.1	6.4-8.4
Length upper labial furrow	1.5	1.5-2.0	1.2	1.1-1.2
Length lower labial furrow	0.9	0.8-1.4	0.9	0.6-1.1
Diameter spiracle	0.6	0.4-0.8	0.5	0.2-0.6
Distance, spiracle to eye	0.7	[1]0.7-1.1	1.1	[1]0.7-1.2
Fin measurements:				
Base first dorsal	8.9	8.9-11.2	9.4	8.3-11.6
Height first dorsal	6.0	6.0-7.8	7.8	7.0-8.8
Base second dorsal	8.9	8.0-9.7	7.3	7.3-9.1
Height second dorsal	4.8	4.5-6.1	5.5	5.2-6.5
Base anal	6.0	5.5-6.6	5.3	4.9-6.4
Height anal	3.0	2.6-3.4	3.5	2.8-4.0
Width pectoral base	4.8	4.7-5.6	4.8	4.5-5.6
Anterior margin pectoral	14.3	12.7-14.3	15.3	13.4-16.6
Overall length pelvics	8.6	[1]8.3-9.9	9.6	[1]8.5-9.6
Upper margin caudal	22.4	20.1-22.4	21.9	20.8-22.7

[1] In 4 males or 6 females.

Mouth width about two-thirds of width of head at mouth corners; edge of lower jaw convex.

Teeth show modest gradient heterodonty; from symphysis to mouth corner teeth become lower relative to their root lengths; tooth size changes also along this gradient, starting from small to largest in about four rows from medials and gradually becoming smaller towards ends of dental band.

Body moderately slender, trunk rather high anteriorly, almost humped above pectorals, sloping posteriorly to pelvics and caudal origins; caudal peduncle long, slender, subquadrate in cross section, a weak postdorsal ridge extending medially from just after second dorsal to caudal origin; an ill-defined predorsal ridge extending for a short distance anterior to first dorsal fin; distance from snout tip to cloaca somewhat less than distance from cloaca to caudal tip.

Dermal denticles small, those of dorsal surface below first dorsal fin base 0.04 to 0.08% of total length in two specimens, 0.15 to 0.35 mm in 457-mm female; anterior edges and posterior cusps of adjacent denticles somewhat overlapping, skin visible between denticles; denticles transparent, without pigment and more or less invisible when wet; chromatophores of skin between denticle bases visible through denticles; bases of denticles short, subquadrate, with relatively short pedicels; medial ridge of crown not subdivided longitudinally (Figure 6); concave depression present on either side of medial ridge, with its lateral boundary deflected slightly outward to form an incipient lateral ridge that may or may not terminate in a short lateral cusp; lateral cusps generally absent from denticles of caudal and ventral surfaces; denticles below first dorsal becoming wider relative to their lengths with increase in specimen size; small denticles

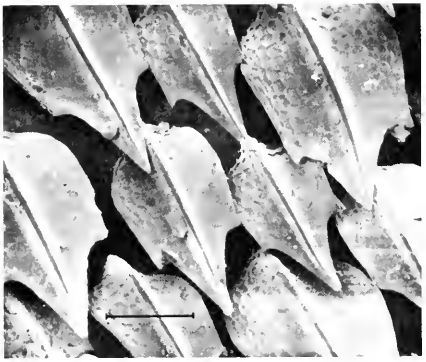

FIGURE 6.—*Iago omanensis.* Dermal denticles from side of trunk below first dorsal fin, 582-mm female, scanning electron microscope photomicrograph, scale line at lower left equals 100 μ.

(less than 0.1 mm long) present on anterior part of palate and inner surfaces of branchial arches, also irregularly and rather sparsely scattered on tongue.

Pectoral fins broad, subangular, with their anterior margins convex, apexes rounded, posterior margins slightly convex, free rear tips rounded, and inner margins convex, relatively short, their anterior margins about an eye diameter shorter than distance from snout tips to first gill opening; pectoral inner margins long, about 1½ to 1⅔ times pectoral base length; posterior margin about 1⅓ to 1½ times in anterior margin; free rear tip of pectoral varying in position from below posterior end of first dorsal base to last third of first dorsal base.

Pectoral fin skeleton, as studied on radiographs, somewhat similar to that of *Galeorhinus;* propterygium with 1 radial, mesopterygium with 3 or 4, segmented metapterygial axis with 10 to 12; metapterygial axis elongate, much larger than anterior basals, with a distal set of segments; radials mostly divided into three segments (proximal, intermediate, and distal), intermediates shorter than proximals or distals, which are equal in length.

Pelvic fins somewhat larger than anal but smaller than second dorsal in area; pelvics in some males relatively smaller than those of females; pelvics triangular, with anterior margins slightly convex to nearly straight, apexes broadly rounded to subangular, posterior margins nearly straight, free rear tips acute (slightly attenuate in some specimens), inner margins straight; pelvic anterior margins 2.6 to 2.8, posterior margins 1.7 to 1.9, and inner margins 1.4 to 1.6 in comparable margins of pectorals.

Claspers and associated secondary sexual structures of males generally similar in basic plan to those described for *Galeorhinus galeus* (as "*Galeus vulgaris*") by Leigh-Sharpe (1921), but differing in several details; claspers long, more slender and more angular distally than those of *Galeorhinus* with bluntly pointed, flattened tips (Figure 5A); claspers of adult males extending well beyond free rear tips of pelvics; clasper groove roofed over and closed by its overlapping sides from apopyle to hypopyle; small pseudosiphon present mediodorsally, its pouch extending anteriorly on clasper. Unlike *Galeorhinus*, the pseudosiphon aperture is much less prominent and is located relatively farther from the clasper tip. Cover rhipidion very large (scarcely developed in *Galeorhinus*), formed as a rounded flap completely covering rhipidion; rhipidion evenly rounded (wedge-shaped in *Galeorhinus*); pseudopera present, dorsolateral and opposite to the rhipidion edge (as in *Galeorhinus*); unlike *Galeorhinus*, the pseudopera is partially covered by another flap, here termed the exorhipidion, which originates laterad to the pseudopera and extends posteriorly to cover part of the rhipidion. Hypopyle opening at level of pseudopera, cover rhipidion, and anterior third of rhipidion.

Clasper skeleton studied from radiographs of six males. Terminology is modified from Jungersen (1899) and White (1936, 1937). One basal cartilage connecting clasper cartilage to pelvic basipterygium; a small beta cartilage present at the junction of basal cartilage and clasper cartilage; details of terminal cartilages not clear, but at least two terminals, a dorsal and a ventral, are present; clasper cartilages heavily calcified in adult males.

First dorsal fin triangular with height much less than length from origin to free rear tip; origin ill-defined, grading into predorsal ridge; anterior margin slightly concave basally but convex towards fin apex, with a 45 degree slope relative to body axis; apex acutely rounded, posterior margin somewhat concave, free rear tip slender, elongate, acute; base much longer than fin height, inner margin about 60 to 70% of fin height; end of first dorsal base about over adpressed apex of pectoral; pectoral free rear tip anterior to pelvic origins by a distance nearly or quite equal to lengths of pelvic bases.

Second dorsal fin generally similar in shape to first dorsal; its height about half length from origin to free rear tip; fin base about 1.4 to 1.5 times height; inner margin about 0.5 to 0.7 of height; origin of second dorsal posterior to midpoint between anal origin and posterior end of pelvic base; free rear tip of second dorsal opposite or slightly posterior to that of anal; second dorsal over twice area of anal.

Anal fin a low triangle, with height about 0.4 in length, anterior margin broadly convex, apex rounded, posterior margin moderately concave, free rear tip slender and acute, and inner margin concave; inner margin almost or quite equal in length to height; fin base 1.4 to 1.6 times fin height.

Dorsal margin of caudal nearly straight, preventral margin broadly convex, and junction of preventral and postventral margins rounded; postventral margin long, concave anteriorly but nearly straight posteriorly and curving abruptly upward into subterminal notch; subterminal margin nearly straight, terminal margin invariably frayed but apparently moderately concave.

Vertebral counts given in Table 3.

Vertebral calcification pattern was studied from transverse sections and radiographs of centra from below first dorsal fin. Terminology for vertebral parts follows Ridewood (1921). Primary double cone without diagonal calcified lamellae; solid dorsal, lateral, and ventral intermedialia present, separated by uncalcified areas for the basidorsals and basiventrals (Figure 3C); notochordal canal at constricted portion of double cone unusually large (as in many other deepwater sharks, a feature possibly correlated with habitat).

The chondrocranium was dissected out in one specimen but is not described here. It is similar in structure to the crania of *Galeorhinus* and *Mustelus* described by Gegenbaur (1872) but differs in numerous details from both.

Stomach very large, subdivided into a sack-like fundus and a long slender pylorus. The fundus extends posteriorly over two-thirds the length of pleuroperitoneal cavity, then reverses direction as the pylorus to continue anteriorly nearly to root of liver, where it joins the spiral intestine. The latter is fusiform, with a spiral valve of about five turns (Figure 3D). The narrow rectum has a slender rectal gland attached distally to the epigonal organ in both sexes. Liver only moderately large, with paired lateral lobes concealing small medial lobe, posterior ends of lateral lobes extending only one-half to two-thirds of distance to posterior end of pleuroperitoneal cavity. Spleen elongate, not nodular, originating dorsally on distal end of fundus and coursing anteroventrally on pylorus to spiral intestine, where it extends posteroventrally to below the first intestinal valve. Pancreas elongate, single, located anterior to spiral intestine and dorsal to stomach. Ovaries well-developed only on right side, with long epigonal organ extending posteriorly to rectal gland; both oviducts well-de-

TABLE 3.—Vertebral numbers in male and female *Iago omanensis*.

Monospondylous precaudals	Diplospondylous precaudals	Caudal vertebrae	Total vertebrae
	Males		
34	48	48	130
34	47	48	129
36	49	58	143
36	47	52	135
37	49	51	137
37	45	51	133
37	51	44	132
	Females		
37	51	50	138
37	47	50	134
37	51	58	146
37	51	52	140
37	51	50	138
38	51	51	140
38	51	51	140
39	52	53	144
42	49	56	147

veloped and functional in all adult females examined, with small nidamental glands almost obsolete on the right side in some specimens; both testes apparently functional, subequally developed in three males examined, with a single epigonal organ attached to left testes. Semilunar valves of conus arteriosus in two rows, the anterior one with three valves, the posterior with three much smaller valves located each on the posterior base of an anterior valve.

Color brownish or grayish above and lighter below, with no conspicuous markings or abrupt color changes from dorsal to ventral; expanded chromatophores in the darkest specimen give a peppered appearance; small areas of darker pigmentation present near tips of both dorsal and caudal fins and in some specimens extending along leading edges of fins; lining of buccal cavity and peritoneum whitish.

VARIATION

The variation in morphometrics among our 16 specimens is substantial, unusually so for a series of adult sharks. Most of the differences do not follow sex, but it is apparent that the abdominal section is longer in females than in males. Thus the distance between pectoral and pelvic bases ranges from 13.7 to 16.1% of total length in seven males but is 16.8 to 20.0% in nine females. This is similar to the situation reported for the squaloid *Euprotomicrus bispinatus* by Hubbs, Iwai, and Matsubara (1967) and in *Carcharhinus leucas* by Thorson, Watson, and Cowan (1966). Large variations in tooth row and vertebral counts were noted also. Despite the range of variation between individuals and the sexual dimorphism in our sample, we find nothing to indicate that more than one species is represented or that the variation can be attributed to known geographical or environmental influences.

REPRODUCTION

One 410-mm specimen in our series has partially developed and uncalcified claspers but has eggs with very early embryos in the oviducts. Thus the specimen is, at least functionally, a female. Histological examination of the ovaries was not made, but gross examination revealed one ripe ovary of normal appearance but little development of the other gonad. A similar instance of the partial development of claspers by a functional female *Centrophorus lusitanicus* was reported by Cadenat (1960). A more extreme example, recorded by King (1966), was of a hermaphroditic *Scyliorhinus canicula* with a single immature clasper, a ripe ovotestis (with ovarian follicles at all stages and seminiferous tubules with mature sperm), and functional nidamental glands, oviducts, vasa deferentia, and seminal vesicles (with sperm). King also listed another *S. canicula* specimen with two immature claspers, a ripe ovotestis, and oviducts, but no seminal vesicles and vasa deferentia. The opposite condition was found in a field-dissected specimen of *Mustelus higmani* by Dr. John Thompson (Springer and Lowe, 1963). This individual lacked claspers but had a pair of enlarged testes.

It may be significant that in the above cases the size of each shark was within the range of its functional sex at maturity regardless of external characters belonging to the opposite sex. The *Iago* and *Centrophorus* females with claspers were larger than would be expected for mature males of the species, but the clasperless male *Mustelus* was smaller than mature females of its species. Both hermaphroditic *Scyliorhinus* were the size of adult females of their species despite the presence of claspers.

Our smallest male, 224 mm long, is immature with uncalcified claspers but six others from 295 to 363 mm are mature. We did not examine internally a 358-mm female, the smallest of its series, but eight others from 395 to 582 mm are mature and have eggs in their oviducts. The eggs are for the most part not large, having yolks not more than 10 mm in diameter, and in our specimens, embryos, when present, are in a very early stage of development. In the oviducts each egg is encased in a thin and soft membranous shell which closely adheres to the oviduct lining. The nidamental glands vary in size from scarcely visible enlargements of the anterior oviduct to about 10 mm in diameter,

but all are far smaller than those present in oviparous scyliorhinids. The condition of nidamental glands and eggshells indicate that *Iago omanensis* is livebearing, with oviductal egg counts suggesting a litter of 2 to 10 young. The relatively small size of egg yolks implies that a maternal source of nourishment is provided the embryos unless the young are extremely small at birth.

SIZE

Iago is one of the smaller carcharhinids. In the Carcharhinidae, *Scoliodon*, the *Protozygaena* group in *Rhidoprionodon*, and *Mustelus* have species nearly or quite as small as *I. omanensis*, though *Eridacnis* species are even smaller. One of the latter, *E. radcliffei*, is apparently the smallest carcharhinid and one of the smallest sharks, with males mature at 186 mm and females at 216 mm.

Size disparity between the sexes is a common phenomenon among elasmobranchs, in all known cases with females larger than males. In *Iago omanensis* this disparity is very marked; our largest male (365 mm) was only 63% as long as the corresponding female (582 mm) and weighed but one-sixth as much.

FOOD

Stomachs of two specimens contained remains of unidentified fish, in one a fish head 32 mm long and in the other a 50-mm section of the posterior trunk of a fish estimated to have been more than 200 mm long.

DISTRIBUTION

Table 1 shows the distribution of 16 of 17 known specimens of *Iago omanensis*, all except the holotype from IIOE Cruise 4B. Only three other shark specimens, all *Mustelus* sp., were collected during Cruise 4B from 81 trawling stations in the northern Arabian Sea. This total of only 19 shark specimens of two species is much lower than the expected catch for comparable gear in many other areas of continental shelf and slope.

A possible explanation for the low incidence of sharks in the catches lies in frequent presence of poorly oxygenated water near the bottom along the coast between the Gulf of Kutch and the Gulf of Oman (See Banse, 1968, for a general account of the hydrography of part of this area). Sharks of species commonly held in marine aquaria are thought to require a high dissolved oxygen level for survival although studies to verify this for particular species have not been made.

Low oxygen concentration in water at the bottom, 0.22 to 0.77 ml/liter, is associated with five of the six IIOE stations at which *Iago omanensis* was taken. It appears that this species may be exceptionally tolerant to low oxygen levels, even at the moderately warm (16.24° to 22.39° C, or about 61.3° to 72.4° F) water it apparently inhabits. In the Red Sea, Marshall and Bourne (1964) reported that their unidentified carcharhinoid (which may be *Iago omanensis* or a close relative) occurred at depths down to 2195 m. As this area and these depths may have oxygen concentrations lower than 1 ml/liter at the end of summer (Richards, 1957), the Marshall and Bourne shark may be able to survive oxygen levels as low as known *Iago omanensis* apparently does in the Arabian Sea.

Gibbs and Hurwitz (1967) regarded the greater development of gill lamellae in the stomiatoid fish, *Chauliodus pammelas* compared with that in *C. sloani* as an adaptation to the low oxygen habitat of *C. pammelas*. We looked at structures having respiratory functions in *Iago omanensis* but found nothing to suggest such an adaptation. *I. omanensis*, however, has no closely allied species as a basis for comparison.

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UPTAKE, ASSIMILATION, AND LOSS OF DDT RESIDUES BY *Euphausia pacifica*, A EUPHAUSIID SHRIMP

ABSTRACT

JAMES L. COX[1]

Euphausia pacifica Hensen, an abundant euphausiid shrimp from the California Current, can acquire sufficient DDT residue from its food to account for amounts found in its tissues. Assimilation efficiencies for DDT in ingested food are similar to published figures for assimilation of carbon from food. The concentration vs. size function suggested by gas-liquid chromatographic analyses of DDT residues in *E. pacifica*, however, was quite different from the function predicted by a theoretical food assimilation model. Direct uptake of ^{14}C-DDT from water was rapid and partially reversible by returning animals to unlabelled flowing seawater. Uptake equilibrium was reached within 72 hr for smaller animals (<3 mg dry weight); larger animals apparently equiliberated after a longer period. ^{14}C-DDT present in animals after 2 weeks exposure to unlabelled flowing water was retained in higher amounts in larger animals (>3 mg dry weight). The possible effects of dietary changes, moulting, and surface to volume ratios on observed natural levels are discussed.

DDT and its congeners are manmade substances which have achieved global distribution. This fact has produced widespread concern over their long-term impact in ecosystems and has stimulated efforts to study DDT transport from a systems analysis viewpoint (Harrison et al., 1970). Indirect evidence (Cox, 1970) suggests an accretion of DDT residues in oceanic food chains and underscores the need to produce information about mechanisms and rates of DDT acquisition and loss by plankton organisms. This paper reports the results of an experimental study of the euphausiid crustacean *Euphausia pacifica* dealing with quantitative aspects of DDT acquisition from food and water, rates of loss of acquisition from food and water, rates of loss of acquired DDT, and factors affecting equilibration with the surrounding water.

Euphausiid crustaceans are among the most abundant zooplankters in many oceanic regions. They are the food of commercially important fishes and in general represent an important link of oceanic food chains. *E. pacifica* is the most abundant euphausiid of the California Current. Ponomareva (1954, 1955, 1959, 1963) has summarized behavioral and population data on this species, and Lasker (1966) has made extensive laboratory studies of its feeding, growth, respiration, and carbon utilization.

METHODS AND MATERIALS

Laboratory maintenance of *E. pacifica* has been described by Lasker and Theilacker (1965). Animals were maintained in a 40-liter capacity tub with flowing seawater at 10 to 12° C and fed daily rations of freshly hatched *Artemia* nauplii. Individuals were kept long enough during the course of the experimental work for noticeable growth. Mortality was extremely low after the first day that the animals were kept in the tub.

In direct uptake experiments, ^{14}C-DDT was added in small carrier volumes of ethanol (ca. 100 μliter) to GFC glass fiber filtered seawater (volumes from 1 to 10 liter) under constant stirring from a magnetic stirrer. Animals were introduced in groups from a small net or turkey baster. At the completion of an uptake run, animals were removed, rinsed briefly with fresh water, and placed in a desiccator for 6 days at

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room temperature. Losses of ^{14}C-DDT during desiccation were insignificant. Dried animals were removed, weighed on a Cahn electrobalance to ± 0.01 mg, placed in scintillation vials with equal volumes of NCS solubilizer (Nuclear-Chicago), and digested 1 hr at 70° C before introduction of scintillation fluid and subsequent counting on a Nuclear Chicago Unilux II scintillation counter.[2]

Loss experiments were done by taking labelled animals, subsampling them for initial ^{14}C-DDT levels, and placing them back in a flowing seawater tank. Water in the tank had a turnover time of less than 10 min, so lost ^{14}C-DDT was rapidly removed from the system. Groups of animals were removed from the tank at intervals and analyzed as described above.

In addition to work with ^{14}C-DDT, freshly caught *E. pacifica* were processed and analyzed for naturally occurring levels of DDT residues according to published methods (Cox, 1970), except that whole euphausiids were ground in the homogenizer, rather than algae on filters.

All direct uptake work was done at concentrations less than 33 ppt (parts per 10^{12}) ^{14}C-DDT in seawater, ranging down to 5 ppt. In uptake and loss experiments, individual samples were taken by removing about 10 to 15 animals from the experimental system, processing them, and plotting the results on log-log (full logarithmic) paper and fitting a least-squares regression line to the logarithmically transformed data. Depending upon the extent of the dry weights of the animals taken in each of the described groups, points corresponding to 1.0, 2.0, 3.0, or 10.0 mg dry weight were taken from the regression line for comparisons.

RESULTS

UPTAKE

Since the lipid constituents of planktonic organisms are not in direct contact with seawater, it is necessary to postulate a two-step process of uptake of DDT residues—first, adsorption on-

[2] Reference to trade name in this publication does not imply endorsement of commercial products by the National Marine Fisheries Service.

to surfaces in contact with seawater and second, diffusion or transport of the adsorbed residues into the lipid constituents of the organism. Initial uptake by *E. pacifica* was rapid; Figure 1 shows the results of a 2-hr uptake experiment. Approximately equal numbers of animals were added to two 7-liter jars containing ^{14}C-DDT at a low ppt concentration. Two hours later, animals were removed and analyzed. The concentration vs. dry weight functions were found to be exponentials, yielding a straight line on the

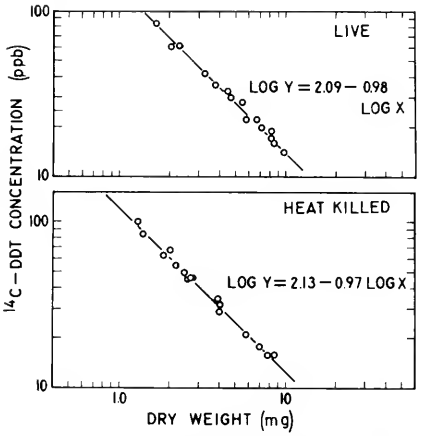

FIGURE 1.—Uptake of ^{14}C-DDT by *Euphausia pacifica* of different weights after 2 hr of exposure to labelled medium.

log-log plot. Initial uptake appeared to be unrelated to the animals' activity or respiration since heat-killed animals had the same total uptake as live animals. The amounts of ^{14}C-DDT taken up per animal were almost identical in these experiments (exactly equal amounts would yield a slope of —1 in the regression function).

In a different series of experiments, the slopes of the log-log concentration vs. dry weight functions changed from —1.05 at 2 hr and —0.99 at 8 hr to —0.67 at 24 hr. This change resulted from increased uptake by larger animals after longer exposure. Figure 2 summarizes the over-

all patterns of uptake for the 72-hr period. Three arbitrary dry weights of animals (2.0, 3.0, and 10.0 mg) were chosen to illustrate different weight effects during uptake. The points corresponding to these dry weights were taken from regression lines like those shown in Figure 1. The values on the ordinate were converted from concentration to total picograms ($g \times 10^{-12}$) of ^{14}C-DDT. After 72 hr of exposure, the 10-mg animal did not reach equilibrium; the 2 and 3 mg animals did reach equilibrium after 72 hr.

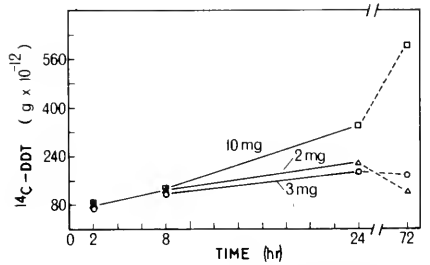

FIGURE 2.—Uptake of ^{14}C-DDT by *Euphausia pacifica* in a closed system. Equilibrium concentration of ^{14}C-DDT in the water was 20 parts per trillion. The three dry weight values were taken from log-log regression lines for subsamples of 10 animals or more. See text for details.

EFFECT OF TEMPERATURE

Temperature appeared to have little effect on initial uptake rates. The Q_{10} for short-term (2 hr of exposure) uptake between 5° and 15° C for an animal of a given dry weight was computed by comparing log-log regression functions for two groups of animals exposed to the same nominal concentrations of ^{14}C-DDT in the medium—one group at 5° C and the other at 15° C. This procedure yielded a Q_{10} of 1.11 for an animal of 2.0 mg dry weight and a Q_{10} of 1.29 for an animal of 10.0 mg dry weight. Both figures suggest a physical process as the limiting step for direct uptake of DDT; the higher figure for larger animals may reflect a higher Q_{10} for transfer into the lipid reservoir of the larger animal.

Del Nimmo (personal communication, 1970) has evidence that DDT residues are transported to internal sites of accumulation by a protein fraction in the haemolymph of penaeid shrimps. If *E. pacifica* is comparable in this regard to the penaeid shrimp, then transport of DDT in the circulatory system must not be the rate-limiting step in uptake, since circulatory rates may be expected to have a higher Q_{10} than those found. Respiratory rates, which are directly dependent upon circulatory rates, exhibit Q_{10} values in excess of 2.2 in *E. pacifica* (Paranjape, 1967).

CONCENTRATION FACTORS

The short-term uptake concentration factors (the ratio of the concentration of DDT in the animals to the concentration in the water after brief exposure) for ^{14}C-DDT changed little over the range of concentrations employed. Table 1 summarizes data that were taken from log-log plots for animals of 1.0 and 3.0 mg dry weight. It is evident that short-term uptake of DDT for an animal of a given size is proportional to the concentration of the DDT in water.

TABLE 1.—Concentration factors after 2 hr exposure.

Equilibrium concentration of ^{14}C-DDT in seawater	Concentration factor × 103 Concentration in animal (dry): concentration in water (w/v)	
Parts per trillion	*1.0 mg*	*3.0 mg*
5	4.4	1.1
[1]20	3.2	1.1
26	4.1	1.2
33	4.1	1.2

[1] This includes data from one-half hour run.

LOSS

If short-term exposure to DDT in the seawater medium of *E. pacifica* results in surface adsorption, one expects that these adsorbed residues will be lost to the medium if the ambient concentration of the DDT is lowered. If all the labelled DDT in a short-term experimental exposure is adsorbed, the animals would be expected to lose eventually all of their label when returned to unlabelled flowing seawater.

Figure 3 shows the results of 2 weeks of "rinsing" on animals originally exposed to ^{14}C-DDT for 2 hr. The lower data points show that a fraction of the ^{14}C activity was retained, although the size vs. estimated ^{14}C-DDT concentration function was altered considerably by the treatment. Figure 4 shows a loss curve constructed from a series of log-log plots such as those in Figures 1 and 3. The 10.0 mg animal apparently neared equilibrium at the end of the 2-week period, but the 2.0 and 3.0 mg animals were still declining. Presumably, the ^{14}C-activity loss occurred by diffusion of the parent compound (^{14}C-DDT) or metabolites into the flowing seawater medium. Some loss may have occurred through moulting. Unfortunately, the conditions of the experiment did not allow any record of moult production.

ASSIMILATION FROM FOOD

Animals were isolated in Carolina dishes and kept at 10° C in the dark in 200 ml of GFC filtered seawater and fed known numbers of freshly hatched *Artemia* nauplii previously labelled with ^{14}C-DDT ($2.7 \pm 0.02 \times 10^{-12}$ g ^{14}C-DDT/nauplius, on the average for groups of 10 to 50). After 24 hr, animals were removed to new dishes and fed daily rations of unlabelled nauplii to ensure flushing of the undigested remains of the labelled nauplii from the guts of the experimental animals. After 2 days, the animals were removed, rinsed, dried in a desiccator, and weighed. Amounts of ^{14}C-DDT activity retained were computed by measuring the activity of the dried animals as described in the section on methods. Amounts of labelled nauplii eaten were calculated by counting the numbers left in the dishes after the 24-hr feeding period. Table 2 summarizes the results of the experiment.

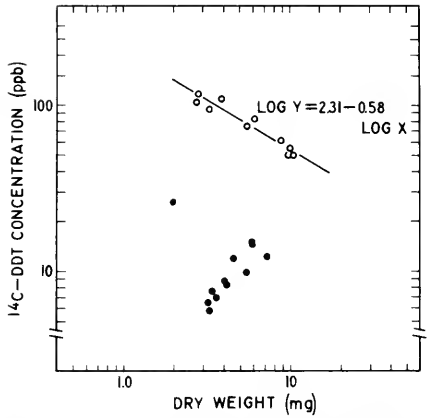

FIGURE 3.—Loss of ^{14}C-DDT from *Euphausia pacifica* kept in a flowing water system. Values for the different dry weights were obtained as indicated in the methods section of the text. The solid dots indicate ^{14}C-DDT concentrations after 2 weeks of exposure to unlabelled flowing seawater. The open dots are for animals exposed to ^{14}C-DDT for 2 hr, then "rinsed" in the flowing seawater system for 2 hr before sampling.

Animal 5 may have had a higher assimilation efficiency because of delayed excretion of the gut contents, presumably attributable to the post-moult condition, i.e., passivity and lack of feeding or swimming movements (Paranjape, 1967). Consequently animal 5 was excluded from further calculations. Animal 1 may have had a lower assimilation efficiency because some loss of labelled material with the moult. The average ^{14}C-DDT assimilation efficiencies for animals 2 to 4 is only slightly lower than Lasker's (1966) estimates of carbon incorporation efficiency for *E. pacifica*.

TABLE 2.—^{14}C-DDT assimilation from labelled *Artemia* nauplii by *Euphausia pacifica*.

Animal	Nauplii eaten / nauplii offered (labelled)	Percent consumption	Amt ^{14}C-DDT ingested picograms ($\pm \times 10^{-12}$)	Amt ^{14}C-DDT assimilated picograms ($\pm \times 10^{-12}$)	Moult[1]		Percent assimilation efficiency
					Pre	Post	
1	30/30	100	81	28	−	+	34
2	44/53	83	119	70	−	−	58
3	38/38	100	103	81	−	−	78
4	49/75	65	132	106	−	−	80
5	21/62	34	62	58	+	−	93

[1] Premoult means moult was recovered after feeding on labelled nauplii. Postmoult means moult was recovered after feeding on unlabelled nauplii.

In another experiment, 12 animals were placed in a vessel and fed ^{14}C-DDT labelled nauplii for 1 hr. Six animals were taken and processed for ^{14}C activity, and the remaining six were allowed to feed on unlabelled nauplii for 2 days before they were processed. The assimilation efficiencies were computed as a ratio of ^{14}C activity in the animals processed after 2 days to the ^{14}C activity in the animals processed immediately after the 1-hr feeding period. This method yielded an assimilation efficiency of 76%.

For calculations, I took a mean of the first four animals' assimilation efficiencies. It is uncertain whether this figure (62%) adequately reflects the influence of moulting on DDT assimilation efficiency. Moulting probably plays an important role in DDT loss from the organism; DDT incorporated into the moult is lost when the moult is shed.

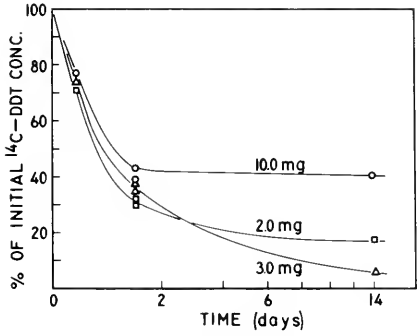

FIGURE 4.—Loss curve constructed from data such as that presented in Figure 3. See text for details.

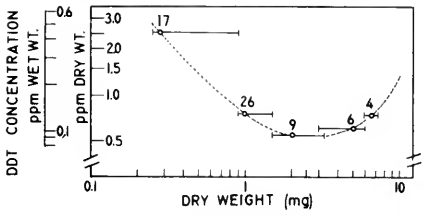

FIGURE 5.—DDT residue concentrations in different sizes of *Euphausia pacifica*. The numbers next to the data points indicate the numbers of animals in the pooled sample analyzed; horizontal brackets indicate the range of weights of individual animals within the groups.

NATURAL LEVELS OF DDT

Figure 5 shows the results of gas chromatographic analyses of *E. pacifica* samples collected in August 1970, the same time that most of the experimental animals were collected. On the basis of DDT acquisition from food, a rising trend in the DDT residue concentrations would be expected as animals grew and aged. In order to examine the discrepancy between the observed DDT values and that which might be expected from cumulative assimilation of DDT residues from food, a model was constructed.

Woodwell, Wurster, and Isaacson (1967) found 0.04 ppm in plankton hauls from a polluted estuary; I have found 0.25 ppm in large, pooled samples of copepods from Monterey Bay. The mean weight of these copepods, 0.95 mg, was only slightly higher than for those eaten by *E. pacifica*. *E. pacifica* also feeds on phytoplankton. The concentrations of phytoplankton, when the density of the standing crop of phytoplankton is high enough to stimulate feeding, are probably below 0.1 ppm, wet weight (Cox, 1970). An intermediate figure can be taken as representative of the DDT concentration of the food of *E. pacifica*. I chose 0.1 ppm as the mean concentration of DDT residues in food.

Employing the carbon budget parameters published by Lasker (1966) and the estimate of DDT residue concentration in food organisms, I calculated the cumulative DDT content of the ingested food of animals of three different dry weights (Table 3). The computed values are compared with values interpolated from the arbitrarily drawn dotted line in Figure 5.

Two conclusions may be drawn from a comparison of columns 7 and 8 in Table 3. First, the estimated values are close enough to the observed values to indicate that ingestion is a sufficient source of DDT residues in *E. pacifica*. Second, the concentration vs. size function of the observed values is quite different from that of the calculated values, indicating that processes other than simple accumulation of a fraction of

TABLE 3.—Calculation of expected DDT residues in different sizes of *Euphausia pacifica*.

Dry weight	Equivalent weight carbon	Carbon growth incorporation efficiency[1]	Cumulative amount nauplius carbon required	DDT equivalent[2] ($g \times 10^{-9}$)	Assumed DDT incorporation efficiency	Parts per 10^6 — dry	
						Expected DDT concentration	Observed DDT concentration
mg	*mg*		*mg*				
1.0	0.42	0.30	1.4	1.4	0.62	0.9	0.75
2.0	0.84	0.15	4.2	4.2	0.62	1.3	0.55
3.0	1.26	0.10	8.4	8.4	0.62	1.7	0.56

[1] Carbon growth incorporation efficiencies were taken from Table 2 of Lasker (1966), the figures shown are not means of the values presented by Lasker but are round-figure approximations which take account of the trends shown and of the different range of sizes of animals used in the laboratory experiments which yielded these figures.
[2] The DDT equivalent of the food was calculated from nauplius carbon assuming a wet weight DDT concentration in the food of 0.1 ppm, and a carbon weight to wet weight ratio of 0.1.

ingested DDT determine DDT residue concentrations in *E. pacifica*.

DISCUSSION

For *E. pacifica*, there are two important sources of DDT residues—direct uptake from water and assimilation from food. Short-term direct uptake is rapid and appears to be at least partially reversible, suggesting adsorption of DDT to exposed surfaces. Over longer periods, these initially acquired residues are transferred to internal deposition sites. The long-term uptake and loss experiments show that larger animals tend to retain more of the initially acquired DDT, possibly because of greater lipid content. Direct uptake from water is a possible mechanism for accumulation of residues if the initially adsorbed residues are continually transferred to internal deposition sites. The rate of initial uptake will depend upon the concentration in seawater (Table 1); retention of these initially acquired residues apparently depends on other factors, judging from the lower set of data points in Figure 3. One determinative factor may be lipid content; values given by Mauchline and Fisher (1969) indicate that lipids, expressed as percentage of body weight, can vary by as much as an order of magnitude in *Euphausia* spp., according to the body weight of the animal. The four lipid values listed by Mauchline and Fisher (1969) for *E. pacifica* correspond closely to the DDT concentration values after 2 weeks rinsing shown in Figure 3. However, in the absence of concurrent lipid values for the animals of the lower data points shown in Figure 3, no conclusion can be drawn about the relationship between retention of ^{14}C-DDT and the percentage lipid composition of the animals. It is reasonable to assume, nonetheless, that the changes in the lipid content of *E. pacifica* which accompany reproductive cycles and seasonal feeding changes will have some impact on the DDT residue content, regardless of the source of the DDT residues.

The second possible source of DDT residues, as previously discussed, is from food. In this case, DDT is almost certainly transported directly in the fat of the food organisms to the fat reservoir of the consumer. Numerous studies indicate that marine organisms do not alter lipids from ingested food (Lasker and Theilacker, 1962; Jezyk and Penicnak, 1966; Jeffries, 1970; and others). Comparison of published values of fatty acid composition for *E. pacifica* (Yamada, 1964) with values for its food, microzooplankton and phytoplankton (Jeffries, 1970), suggests that mass assimilation of fatty constituents along with DDT residues is taking place.

As has been suggested, food is probably a sufficient source of DDT residues in *E. pacifica* (Table 3). Direct uptake may contribute to DDT residues in *E. pacifica*, but its role cannot be assessed because of the lack of seasonal data on DDT concentrations in seawater as well as uncertainties about DDT's availability to organisms in the natural environment (Cox, 1971).

Some basis must be sought to explain the unexpected higher concentrations of DDT residues in the smaller animals. Three possibilities exist: (1) the food of immature *E. pacifica* may have higher DDT concentrations, (2) direct uptake from water is more important for the smaller animals because of their higher area:volume

ratios, or (3) smaller animals have not used any of their lipid reserves, which use may cause loss of some DDT residues. The data presented here do not allow conclusions on the relative importance of these possibilities.

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A KEY TO THE AMERICAN PACIFIC SHRIMPS OF THE GENUS *Trachypenaeus* (DECAPODA, PENAEIDAE), WITH THE DESCRIPTION OF A NEW SPECIES

ISABEL PÉREZ FARFANTE[1]

ABSTRACT

Study of American Pacific members of the genus *Trachypenaeus* reveals that variation in armature of the telson includes not only movable spines, but also fixed spines and even no spines at all. It also confirms that the eighth somite bears two arthrobranchiae instead of one arthobranchia and one pleurobranchia. A new species, *Trachypenaeus fuscina*, is described, the specific features of *T. faoea* Loesch and Avila are presented, and a key to the five members of the genus occurring in the region, together with their ranges, is included.

Along the Pacific coast of Latin America species of *Penaeus* are the mainstay of the shrimp fisheries; however, members of various other genera contribute to the catches in significant quantities. Among the latter, three *Trachypenaeus* have been previously recognized: *T. byrdi* Burkenroad, *T. similis pacificus* Burkenroad, and *T. faoea* Loesch and Avila. A fourth, noncommercial species, *T. brevisuturae* Burkenroad, is also found in the region. Burkenroad (1934a, 1938), presented detailed descriptions of the taxa he described, but the characters cited for *T. faoea*, except color pattern, have not proven to be diagnostic. Since the commercial *Trachypenaeus* are indiscriminately known by the common names of "tigre" and "cebra," a definition of *T. faoea* is needed.

The study of collections of American Pacific *Trachypenaeus* has shown that yet another commercial species of this genus occurs in the area. It also pointed out a previously undescribed variation in the armature of the telson, and confirmed the identity of the gills on the eighth somite.

The measurement of total length is the linear distance from tip of rostrum to posterior end of telson, and that of carapace length is the distance from orbital margin to midposterior margin of carapace. The ratio, length of posteriormost pair of telsonic spines to width of terminal portion of telson, is presented in the following manner: length of spine/width of terminal portion = average ratio (*N*, number of specimens: range of variation).

GENUS *Trachypenaeus* ALCOCK

Trachypeneus Alcock, 1901: 15.—Burkenroad, 1934a: 49.—Burkenroad, 1934b: 73, 94.

Trachypenaeus.—Kubo, 1949: 391.—Dall, 1957: 202.

Type-species by original designation, *Penaeus anchoralis* Bate, 1881.

The telson of the genus *Trachypenaeus* was described by Kubo (1949) as lacking fixed spines, and by Dall (1957) as possessing several pairs of lateral movable spines. Previously, Burkenroad (1934b) had proposed a grouping of the genera of the subfamily Penaeinae in four series; he defined the series *Trachypenaeus* as having a variable number of mobile lateral spines on the telson and characterized the series *Parapenaeus* as possessing one to three pairs of movable spines in addition to a fixed posterior pair, considering the presence of fixed spines on the telson as a unique character for the latter series.

In the species of *Trachypenaeus* described below, however, the posteriormost of the four pairs of spines on the telson is fixed, and in *Trachy-*

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penaeus byrdi Burkenroad the telson lacks spines. The telson of the members of the genus, thus, must now be more broadly characterized as having several pairs of lateral movable spines, or several pairs of movable spines anteriorly and a fixed posterior pair, or unarmed. Furthermore, the evidence presented here indicates that the character of the posteriormost pair of telsonic spines is not a unique character of the series *Parapenaeus*.

The branchial formula of *Trachypenaeus* was given by Dall (1957) as follows: pleurobranchiae on somites IX to XII; a rudimentary arthrobranchia on somite VII, anterior and posterior arthrobranchiae on VIII to XII, and a posterior arthrobranchia only on XIII; mastigobranchiae (epipodites) on VII, VIII and XII [first, second maxillipeds and third pereiopod], sometimes also on X and XI [first and second pereiopods]. All American species possess this combination of branchiae, including epipodites on the first and second pereiopods, and, in addition, a vestigial anterior arthrobranchia on somite XIII.

In *Trachypenaeus*, the anterior arthrobranchia on somite VIII is considerably displaced dorsally, and appears to occupy the position of a pleurobranchia; however, its attachment is on the arthrodial membrane. In Figure 1 the openings of the branchiae on somites VIII and IX, together with the proximal parts of the second and third maxillipeds, are depicted; this figure clearly shows that the two arthrobranchiae on somite VIII are attached to the arthrodial membrane, like those on somite IX, whereas the pleurobranchia on the latter somite has its origin on the pleural membrane.

Burkenroad (1934a; see also 1934b) divided the genus *Trachypenaeus* into two subgenera, *Trachypenaeus* and *Trachysalambria*, the latter possessing epipodites on the first and second pereiopods, and a thelycum with a median pocket on sternite XIV; the former lacks these characters. Later, Burkenroad (1959) observed that some members of *Trachysalambria* lack such epipodites and, thus, questioned the "usefulness" of his division. Recently, a number of species from the Indo-Pacific have been described which bear epipodites on the first two pairs of pereiopods but the thelyca, as indicated by Racek and Dall (1965), differ from that Burkenroad attributed to the members of *Trachysalambria*. Consequently, more investigations are needed to interpret the interrelationships of the species of the genus. It should be pointed out, however, that all American species of *Trachypenaeus* exhibit the characters given by Burkenroad for *Trachysalambria*.

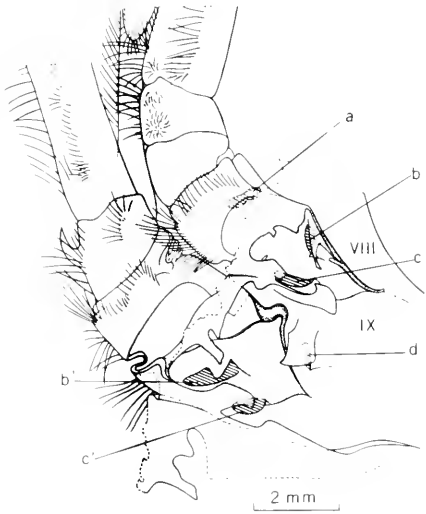

FIGURE 1.—*Trachypenaeus fascina* sp. n., ♀ 33 mm carapace length, off Barra de San Marcos, Chiapas, México. Dorsal view of proximal part of second and third maxillipeds and attachments of gills on arthrodial and pleural membranes of somites VIII and IX (second maxilliped has been displaced laterally). a, Podobranchia. b, b'. Anterior arthrobranchiae. c, c'. Posterior arthrobranchiae. d. Pleurobranchia.

The spelling of the generic name used here, *Trachypenaeus* instead of *Trachypeneus* as was originally published, is based on the decision reached by the International Commission on Zoological Nomenclature, Opinion 864, 1969, Bull. Zool. Nomencl. Vol. 25, Parts 4-5, p. 138-147.

Trachypenaeus fuscina SPECIES NOVA

FIGURES 1, 2, 3A, 4A-F, 5A, 6

"PINTO," "CEBRA," "TIGRE,"

Trachypeneus faoe Lindner, 1957 [part], *nomen nudum*: 48, 49, 81, 145.—Crocker, 1967 [part]: 8, 57.

MATERIAL

Holotype.—♀, USNM 135403, off Cocodrilo, Chiapas, México, 22 m, October 31, 1969, H. Romero and G. Gómez, 35.25 mm carapace length, 135 mm total length, ratio length of spine width of terminal portion of telson = 0.55.

Allotype.—♂, USNM 135404, off La Tapada, Chiapas, México, 22 m, July 31, 1970, D. Palacios, 26 mm carapace length, 108 mm total length, ratio length of spine width of terminal portion of telson = 0.80.

Paratypes.—México. Oaxaca. 8 ♀, IBUNM-USNM, Salina Cruz, May, 1961, E. Martín F. 1 ♂, INIBP, Santa María Xadani, Laguna Superior, July 23, 1970, I. Pérez Farfante. 1 ♀, USNM, off Las Chiches, 24 m, August 6, 1966, Z. Ortiz and G. Gómez. 2 ♂ 1 ♀, USNM, Golfo de Tehuantepec, July 14, 1963, I. Mayés. Chiapas. 3♀, USNM, off Barra de San Marcos, 27 m, March 18, 1964, A. Guerra. 3 ♀, INIBP-USNM, off mouth of Río Suchiate, 7-13 m, February 12, 1968, Romero, Ortiz, Sánchez, and Arias. 1 ♀, USNM, off La Tapada, 7-9 m, February 5, 1968, Romero, Ortiz, Sánchez, and Arias. 4 ♂ 5 ♀, INIBP-USNM, off La Tapada, 22 m, July 31, 1970, D. Palacios. 5 ♀, INIBP-USNM, off Cocodrilo, 22 m, October 31, 1969, H. Romero, and G. Gómez. Ecuador. 1 ♀, USNM, off Playas, September 2, 1962, fishermen. Perú. 2 ♀, USNM, Caleta La Cruz, Tumbes, 70 m, E. M. del Solar.

DESCRIPTION

Carapace pubescent (Figure 2); dorsum densely covered by setae; paired bands of longer setae flanking postrostral carina, from rostrum to various levels in posterior third of carapace; another band on dorsal side of antennal carina; longer setae also along cervical, hepatic and branchiocardiac sulci, and others forming patches on orbital region and posteroventral portion

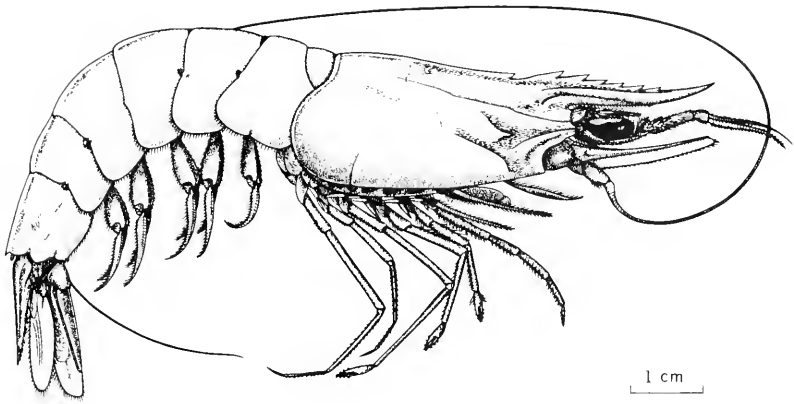

FIGURE 2.—*Trachypenaeus fuscina* sp. n. Lateral view, ♀ 33 mm carapace length, off Cocodrilo, Chiapas, México.

of antennal carina; branchial region covered with short setae; pair of small, bare, crescent-shaped to semicircular areas at anterior end of posterior third of carapace, flanking postrostral carina. Abdomen naked, except for elongate patches of long setae on each side of mid-dorsal carina on third to sixth somites, and two additional paired patches often present on sixth somite, one dorsal and other ventral to cicatrices. Telson (Figure 3A) with two pairs of longitudinal bands of long setae, one along walls of median sulcus, and other along lateral sulci.

Rostral teeth 6-7, first tooth situated immediately behind orbital margin; epigastric tooth at posterior end of anterior fourth of carapace. Rostrum reaching as far as proximal fourth of dorsal flagellum; basal portion ascending well above level of carapace, and apical one-third, unarmed portion, decreasing progressively in height, and curving upward. Adrostral carina slightly sigmoidal, ending about midway between first rostral and epigastric tooth. Postrostral carina strong, long, reaching almost to posterior margin of carapace, higher anteriorly, bearing elongate fossette, immediately behind midlength, and several pits posteriorly. Orbital angle produced into rather broad orbital spine. Gastro-orbital carina and orbito-antennal sulcus absent. Postocular sulcus deep, extending posteroventrally to about level of orbital angle. Longitudinal suture well marked, long, extending along two-thirds of carapace or slightly more. Transverse suture short, clearly distinct, situated at level of coxa of third pereiopod. Antennal and hepatic spines long and strongly acuminate. Antennal carina prominent, extending to below hepatic spine. Cervical sulcus shallow and short, not intercepting longitudinal suture. Hepatic carina and hepatic sulcus well marked, and inclined anteroventrally, their length about one-third that of carapace. Postcervical line sinuous, extending from postrostral carina to near posterior end of hepatic carina. Branchiocardiac sulcus feeble, marked ventrally by obtuse carina. Pterygostomian angle obtuse, its ventral margin sloping posteroventrally before turning backwards.

Antennular flagella subequal, shorter than either antennular peduncle or carapace, slightly longer in males than in females of same length, and proportionally longest in subadult; ratio of flagellar length to carapace length about 0.66 in shrimp of 15 mm carapace length, ratio decreasing with increasing length of shrimp to about 0.40 in shrimp with carapace length of 40 mm. First segment of antennular peduncle with distomedian border produced into heavy, scalelike projection densely covered with long setae; distolateral spine prominent, slender and sharp; prosartema long, extending to distal end of segment; stylocerite attaining midlength of segment.

Antennal flagellum long, almost twice total length of shrimp; scaphocerite reaching distal end of antennular peduncle, elongate, its length 2½ times maximum width; lateral, thickened margin ending anteriorly in strongly pointed spine.

Third maxilliped surpassing carpocerite by as much as dactyl and one-fourth of propodus; first pereiopod reaching, at most, base of carpocerite; second pereiopod surpassing distal end of carpocerite by as much as three-quarters of dactyl; third pereiopod exceeding carpocerite by as much as propodus and one-tenth of carpus; fourth pereiopod extending to about same level as first; fifth pereiopod very long and slender, exceeding carpocerite by entire length of dactyl, and surpassing fourth by propodus and four-fifths length of carpus. Spine on basis of third maxilliped, and, as in all members of genus, on first and second pereipods. Epipodites on first and second pereiopods deeply bifurcate, epipodites on second maxilliped and third pereiopod unfurcate; vestigial anterior arthrobranchia on somite XIII.

Abdomen with middorsal carina extending from posterior half of second to sixth somite, carina low and rounded on second, rather acute on third, and forming high and sharp keel from fourth to sixth somites; fourth and fifth somites with posteromedian V-shaped notch; sixth somite bearing middorsal spine posteriorly, small spine at posteroventral angles, and two cicatrices on each side, anterior one sensibly longer. Telson (Figure 3A) shorter than inner ramus of uropod, with median sulcus deep anteriorly and well marked posteriorly to base of terminal portion; paired rounded carinae flanking median sulcus, and sharp carinae bordering oblique, lateral

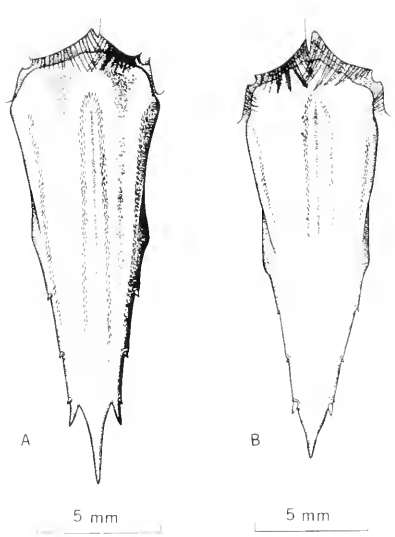

FIGURE 3.—Telsons. A. *Trachypenaeus fuscina* sp. n., ♀ 33 mm carapace length, off Cocodrilo, Chiapas, México. B. *Trachypenaeus favea* Loesch and Avila, ♀ 37 mm carapace length, Ensenada de Garachiné, Golfo de Panamá, Panamá.

sulci; terminal portion (from mesial base of posteriormost spine to apex) from relatively short to long, its length relative to its width at base ranging from 1.60 to 3.70; telson armed with four pairs of lateral spines, posteriormost pair fixed and long, ratio length of spine/width of terminal portion = 0.70 (N 27: 1-0.50); other spines movable and strong, posterior pair (located at lateral base of longer fixed spines) relatively long, anterior two pairs (located posterior to midshoulder of telson) small, but usually visible with naked eye.

Petasma (Figure 4A, B) with large, hornlike, distolateral projections, broad at base, curving laterally, and opening dorsally near anterior margin by long, transverse slit; posteroventral wall of horns reflexed dorsally and produced forward into membranous flap; distomedian projections short, overhanging distoventral aperture of petasma. Ventrolateral lobule of petasma almost entirely cornified, including ventral wall of horns and dorsally reflexed lateral margin; proximomedian portion of lobule with rather flexible strip, tapering to base of distal two-fifths of median margin, there ending where corneous sclerite, curving mesially, reaches margin; soft elliptical area immediately distal to corneous section of margin. Dorsolateral lobule bearing narrow rib along proximomesial third, rib broadening proximally and turning mesially at proximal extremity resembling golf club. Dorsomedian lobule with narrow, distally bifurcate rib at base of distomedian projection, its length about one-third that of lobule. Length of petasma—from apex of distomedian projection to proximal margin of dorsolateral lobule—almost one and one-fifth times its width at level of distolateral projections.

In males, posterior margin of sternite XIII (Figure 4F) bearing large, elongate, subelliptical to ovate median plate, latter with obtuse to acute tip, and numerous marginal setae; anterior half of sternite XIV with strong, subpyramidal prominence, its anterolateral, ventromedially inclined edges often forming shelflike ridges.

Appendix masculina (Figure 4C-E) thick, subcircular in outline, its length along midline subequal to maximum width, and produced into two proximolateral prominences; dorsal wall cornified, except for distal and lateral margins, these, together with ventral wall, rather soft; entire, broad, median border setiferous, setae continuing on distal margin forming narrow band. Dorsal base of endopod with corneous, roughly trapezoidal sclerite, bearing strong median rib articulating distally with appendix masculina; rib with small setiferous depression on distal portion of ventral surface. Accessory sclerite on dorsolateral margin of endopod.

Thelycum (Figure 5A) with posterior part bearing anterolateral, subtriangular, heavily sclerotized projections; anterior part of sternite XIV, with platelike base, projecting as median prominence, and produced forward into pair of lateral, elongate, tonguelike flaps, extending al-

most to anterior margin of median plate; bases of flaps strongly inclined dorsomedially, forming depression limited posteriorly by prominence; margins of flaps often reflexed ventrally. Platelike portion of sternite XIV naked; its lateral margins pronouncedly curving posteromesially, forming deep emarginations with projections from posterior part; basal portions of

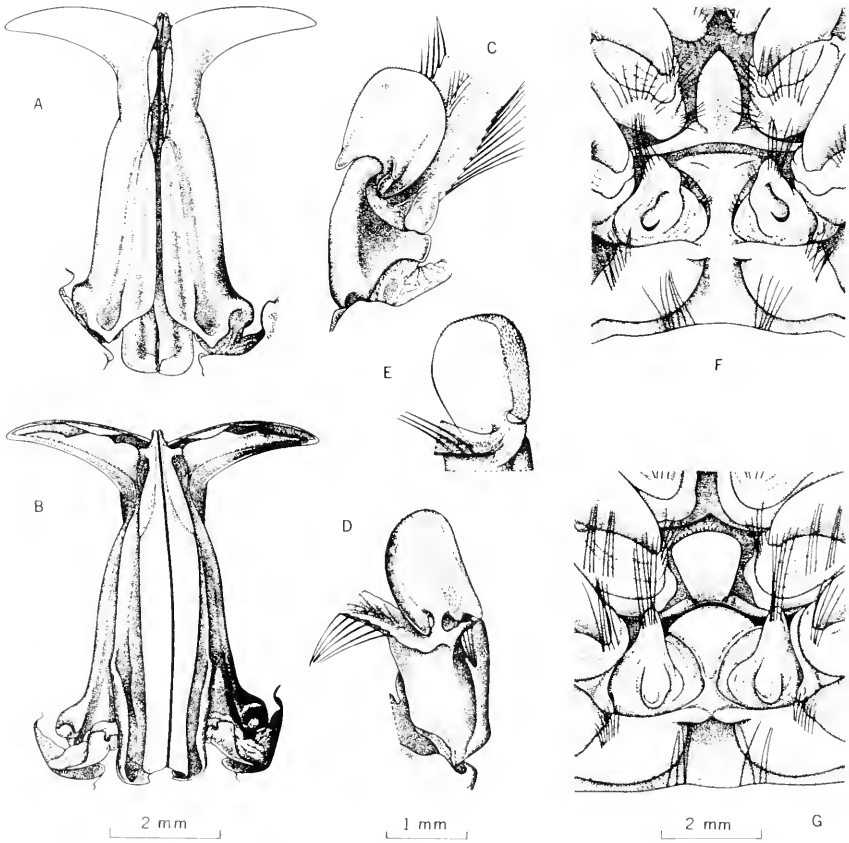

FIGURE 4.—*Trachypenaeus fuscina* sp. n., ♂ 20 mm carapace length, off Cocodrilo, Chiapas, México. Petasma: A. Ventral view. B. Dorsal view. Appendix masculina and proximal portion of endopod: C. Dorsal view. D. Ventral view (endopod displaced). E. Ventromedial view of appendix masculina. F. Sternites XIII and XIV. G. *Trachypenaeus faoca* Loesch and Avila, ♂ 18 mm carapace length, Playa Bella Vista, Panamá, sternites XIII and XIV.

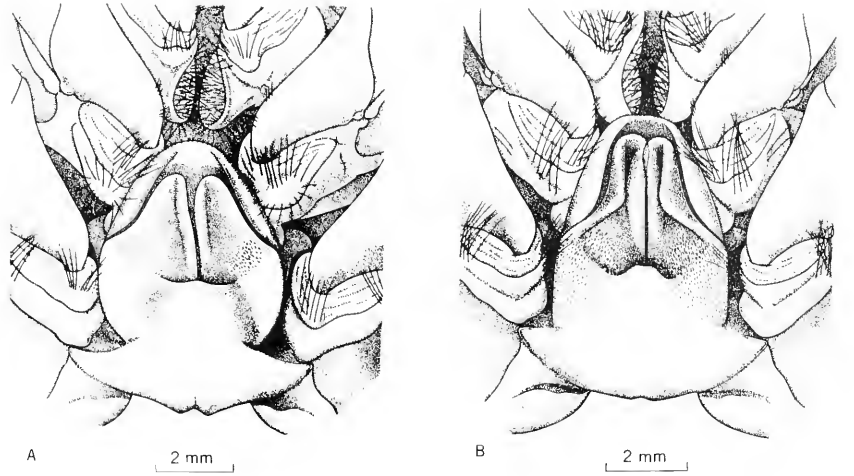

FIGURE 5.—Thelyca. A. *Trachypenaeus fuscina* sp. n., ♀ 34.5 mm carapace length, off Barra de San Marcos, Chiapas, México. B. *Trachypenaeus faoca* Loesch and Avila, ♀ 32.5 mm carapace length, Ensenada de Garachiné, Golfo de Panamá, Panamá.

flaps setose. Median plate of sternite XIII large, oval, strongly concave ventrally, with margin tumid, and bearing setae; plate extending posteriorly joining dorsal wall of flaps, thus giving rise to median pocket; latter bearing paired apertures of internal seminal receptacles laterally (Figure 6B). In impregnated females, median pocket occupied by sclerotized, sperm-free, brownish component of spermatophores, extending almost to anterior margin of median plate. Hardened, glutinous material, extruded with spermatophores, protruding through slit between flaps, forming plug on ventral surface of flaps.

Internal seminal receptacles (Figure 6A, B) consisting of paired, longitudinally arranged, trilobed, membranous sacs: single, large, posterior lobe, dorsal to median pocket, extending caudally almost to posterior margin of sternite XIV; and two small lobes, one directed anteromesially, dorsal to median plate, and the other laterally, dorsal to small hood of sternite XIV.

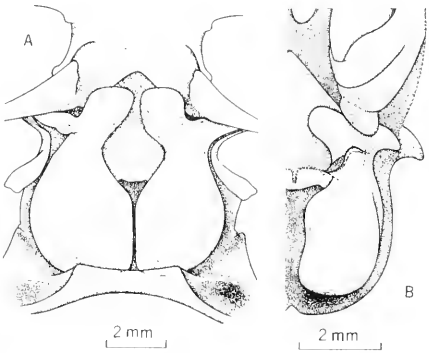

FIGURE 6.—*Trachypenaeus fuscina* sp. n. A. Seminal receptacles (dorsal view), ♀ 39.5 mm carapace length, off Salina Cruz, Oaxaca, México. B. Left seminal receptacle (ventral view), ♀ 31 mm carapace length, mouth of Río Suchiate, Chiapas, México.

Seminal receptacles derived from paired, deep invaginations at anterolateral extremities of sternite XIV. In impregnated females, seminal receptacles enclosing main component of spermatophores, consisting of thin walled sac containing subspherical masses of spermatozoa.

COLOR

Juveniles recently caught in inshore water of Oaxaca, México, light buff with brownish red suffusion; transverse, dark reddish brown bands on posterior part of abdominal somites; bands convex anteriorly, with widest portion on middorsal line, extending ventrally and forming patch on posterior half of pleuron. Preserved adults (fresh ones not observed by me) with distinct dark abdominal bands, similar to those in juveniles.

SIZE

Largest specimen examined, ♀ 40.5 mm carapace length, about 150 mm total length, from off La Tapada, Chiapas, México, depth 7-9 m. Males smaller than females, largest observed ♂ allotype, 26 mm carapace length, 108 mm total length.

DISTRIBUTION

T. fuscina has been found in the Golfo de Tehuantepec, along the coasts of Oaxaca and Chiapas, México, and in the Golfo de Guayaquil, as far south as Tumbes, Perú. Although this species and *T. faoca* are sympatric in the Golfo de Guayaquil, apparently *T. fuscina* ranges farther north, since *T. faoca* has not been found along the southwestern coast of México.

REMARKS

T. fuscina is very closely related to *T. faoca*, but differs from it in several aspects—mainly in features of the telson (Figure 3A). In *T. fuscina* the posteriormost pair of spines is longer, and fixed instead of movable, this being the only member of the genus *Trachypenaeus* with immovable spines on the telson; the anterior three pairs of telsonic spines are stronger than in *T. faoca*, particularly those spines at the lateral base of the posteriormost pair; also the median sulcus is well marked as far as the base of the terminal portion of the telson. In *T. fuscina* the platelike base of the anterior part of sternite XIV in females (Figure 5A) is naked, and its lateral margins curve strongly posteromesially, giving rise to a deep emargination at the junction of the plate with the posterior part of the thelycum (sternite XIV). In males, the median plate of sternite XIII narrows anteriorly, usually tapering to a point (Figure 4F).

It should be pointed out that during copulation in this species, as well as in *T. faoca*, the male transfers to each seminal receptacle a very thin sac containing the spermatozoa, which are grouped into subspherical masses. These masses are not individually transmitted to the seminal receptacles as believed by Burkenroad (1934b), a phenomenon considered by him to be typical of the genus *Trachypenaeus*.

ETYMOLOGY

Fuscina, L., = three-pronged fork—referring to the trifid appearance of terminal portion of telson.

Trachypenaeus faoea LOESCH AND AVILA

FIGURES 3B, 4G, 5B

"CEBRA," "TIGRE," "INDIO," "CARABALI"

Trachypeneus faoe Lindner, 1957 [part], *nomen nudum*: 34, 35, 42, 43, 48, 49, 60, 61, 133, 134. —U.S. Fish and Wildlife Service, 1962: 2, 6.—Croker, 1967 [part]: 8, 19, 30, 39, 47, 57.

Trachypeneus faoea Loesch and Avila, 1964: 4-8, 16, 21, 24-28, fig. 8b, 13b.—Avila and Loesch, 1965: 3, 5, 6, 9, 10, 16, 19, 20, 23, 24, fig. 4b.

Trachypenaeus face.—Food and Agriculture Organization of the United Nations, 1965: 10.

MATERIAL

Neotype.—♀, USNM 135398, Playas, Ecuador, January 7, 1964, Ortiz, 28 mm carapace length, 110 mm total length, ratio length of spine/width of terminal portion of telson = 0.25.

Panamá. 7 ♂ 21 ♀, USNM, Playa Bella Vista, March 20, 1955, M. D. Burkenroad. 3 ♀, USNM, Ensenada de Garachiné, Bahía San Miguel, 16 m, April 18, 1967, *Shimada* Sta. 14. 3 ♀, USNM, 4 km W of Punta Garachiné, 24 m, April 18, 1967, *Shimada* Sta. 78. Colombia. 7 ♀, USNM, Tortugas Grounds, S of Buenaventura, 9 m, September 19, 1969, L. W. Knapp, *Caeique* Sta. LK69-24. Ecuador. 22 ♀, USNM, Playas, January 7, 1964, Ortiz. 1 ♂ 4 ♀, USNM, Boca de Tenguel, April 14, 1966, Ansaldo.

TYPE MATERIAL

Loesch and Avila (1964) did not cite any material in particular; however, as the title of their work indicates, the specimens studied and illustrated in their keys were from Ecuador. Because of the close similarities between *T. faoea* and *T. fuscina* it seems mandatory that a neotype of *T. faoea* be selected, so that no confusion will arise as to the identity of the species of Loesch and Avila. Therefore, a neotype is here designated from a lot of females of *Trachypenaeus faoea* collected in Playas, Ecuador, which was identified and sent to me by the authors of the species. Inasmuch as the combination of specific morphological characters of this species have not been indicated previously, they are presented here.

"*Trachypeneus faoe*" first appeared in the literature in the report of a survey of the shrimp fisheries of Central and South America by Lindner (1957). This author grouped it with *T. byrdi* as one of the "larger zebra shrimp," noting the "blue-black" stripes on the abdomen and citing various other common names. Lindner, obviously, did not intend to describe the species, and did not include any specific character. He reported "*T. faoe*" in the shrimp fisheries of the American Pacific, from México to Perú, including El Salvador, Costa Rica, Panamá, Colombia, and Ecuador. Later (U. S. Fish and Wildlife Service, 1962) the species was cited as one of "medium and small" shrimp found in the catches of Guatemala.

The original description of the species appeared in the keys to the commercial penaeid shrimps of Ecuador by Loesch and Avila (1964), and was mistakenly ascribed to Burkenroad. As stated by Avila and Loesch (1965), Burkenroad had planned to describe the species, but had not done so prior to the publication of their keys, nor has his description appeared since. *T. faoea* was distinguished from other penaeids found in the Ecuadorian catches on the basis of four morphological characteristics and color pattern. The following characters were cited: "No teeth on ventral portion of rostrum. . . . No well-developed dorsolateral sulcus on the posterior part of the last abdominal segment. . . . Backward-pointing dorsal spine on last abdominal segment only. . . . Telson not armed with lateral armature." The first two characters are shared by many species treated in their keys, and are actually supraspecific; the third character is common to all but one (*T. byrdi* Burkenroad) of the American Pacific *Trachypenaeus*, and the fourth is inaccurate: *T. faoea* does possess small lateral spines on the telson.

The color of the species was described as follows: "Tail section light anteriorly and dark posteriorly on each abdominal segment. . . . No distinctive patterns on second and third abdominal segments, each segment similarly colored with wide dark band covering ¾ the width of posterior part of each abdominal segment. More than ½ of color of tail is dark (dark brown)." An entire animal and a separate abdomen were figured. In both illustrations the telson appears unarmed. The figure of the entire animal is accompanied by the rostral tooth formula, $\frac{6\text{-}7}{0}$. Of the characters cited, color is the only one that appears to be typical of the species, and it can only be applied to identification of fresh or recently preserved animals. It is unfortunate that circumstances prevented Loesch and Avila, who dealt with large numbers of specimens, from publishing a detailed description of *T. faoea*.

T. faoea is very closely related to *T. fuscina*, and except for the following, the above descrip-

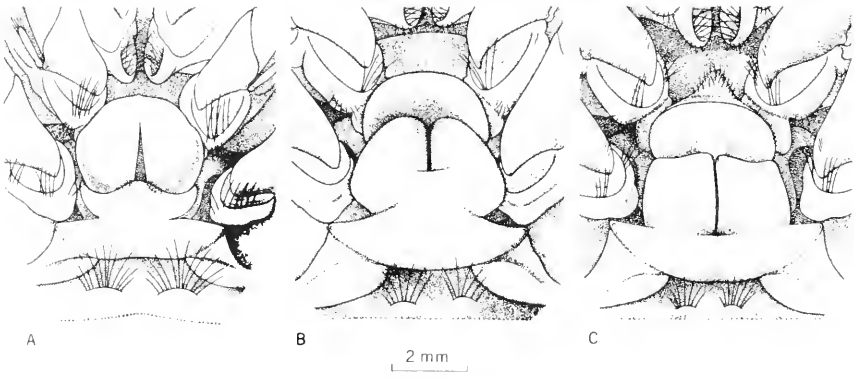

FIGURE 7.—Thelyca. A, *Trachypenaeus brevisuturae* Burkenroad, ♀ 21.5 mm carapace length, off Zacapulco, Chiapas, México. B, *Trachypenaeus byrdi* Burkenroad, ♀ 27 mm carapace length, Golfo de Panamá, Panamá (syntype). C, *Trachypenaeus similis pacificus* Burkenroad, ♀ 25 mm carapace length, Archipiélago de las Perlas, Golfo de Panamá, Panamá (syntype).

tion of *T. fuscina* also applies to *T. faoea*: the armature of the telson, the shape and pubescence of the platelike base of the anterior part of sternite XIV in females, the marginal contour of the median plate of sternite XIII in males, and perhaps color.

In *T. faoea* the posteriormost of the four pairs of spines on the telson (Figure 3B) are movable instead of fixed, and shorter than those of *T. fuscina*, the ratio length of spine width of terminal portion = 0.10 (N 47: 0.75-0.20); the other three pairs of movable spines, including those located at the lateral base of the posteriormost spines, are minute, actually microscopic. In *T. faoea* the median sulcus of the telson is deep anteriorly but hardly perceptible or indistinct posteriorly.

In males of *T. faoea* the median plate at the posterior margin of sternite XIII (Figure 4G) usually varies from subtrapezoidal (widest anteriorly) to suborbicular. In females, the structure of the thelycum (Figure 5B) is similar to that of *T. fuscina;* however, lateral margins of the platelike base of the anterior part of sternite XIV are almost straight, not or barely curving posteromesially, forming about 90° angles with projections of the posterior part; furthermore, the lateral areas of that platelike portion are studded with rather densely set setae, the latter extending onto basal portions of the flaps.

Avila and Loesch (1965) described the color of recently preserved juvenile specimens of *T. faoea* as dark blue or purple with light uropods. They noticed that specimens preserved for 2 days in 5% Formalin exhibit a clear, horse-shoe shaped band on the first three abdominal somites, which is open anteriorly. These observations were based on material from Ecuador and differ from those made by me on *T. fuscina*. Recorded notes on color are so limited that conclusions in regard to differences in this character between the two species must await further observations throughout their ranges.

DISTRIBUTION

Although this species has been reported from as far north as the coasts of Guatemala, El Salvador and Costa Rica, the collections available to me are limited to the area from Panamá to Ecuador. Examination of additional samples of

"cebra," or "tigre" shrimp from Central and South America will be necessary to establish the actual distribution of *T. faoea* as well as that of *T. fuscina*, both known by the same common names.

With the discovery of an additional species of *Trachypenaeus* in the American Pacific, it seems appropriate to present a key to the five members of the genus occurring in the region, together with the range of each.

KEY TO THE AMERICAN PACIFIC SPECIES OF *Trachypenaeus*

1. Carapace with longitudinal suture short, not extending to hepatic spine. Third maxilliped lacking spine on basis; first pereiopod bearing spine on ischium. Thelycum with median plate armed with small anteromedian spine (clearly distinct in juvenile, barely perceptible in adult); anterior part of sternite XIV shorter than posterior part, and not produced as paired flaps (Figure 7A). Petasma with dorsal spinelike projection near apex of horn .. .. *T. brevisuturae* Burkenroad
(From off Punta Arenas, Golfo de California, to El Salvador).

Carapace with longitudinal suture long, extending posteriorly beyond hepatic spine. Third maxilliped bearing spine on basis; first pereiopod lacking spine on ischium. Thelycum with median plate lacking anteromedian spine; anterior part of sternite XIV longer than posterior part, and produced as paired flaps. Petasma without projection near apex of horn 2

2. Spine present on posterior end of middorsal carina of last two, three, or four abdominal somites. Telson unarmed. Thelycum with slit between flaps never reaching posterior part of sternite XIV, and with median plate short, not reaching gonopores (Figure 7B) .... *T. byrdi* Burkenroad
(From Guatemala to Golfo de Guayaquil)

Spine present only on posterior end of middorsal carina of last abdominal somite. Telson armed with lateral spines. Thelycum with slit between flaps reaching posterior part of sternite XIV, if not, with median plate long, reaching, or almost reaching, gonopores .. 3

3. Rostral teeth 7 to 10, usually 8 or more. Telson with proximal triangular patch of long setae on each side of median sulcus. Thelycum with median plate not excavated, short, and protruding ventrally on midportion. Anterior part of sternite XIV lacking platelike base and bearing subrectangular flaps, extending only to posterior part of median plate (Figure 7C) ..... *T. similis pacificus* Burkenroad
(From Bahía Concepción, Golfo de California, to Tumbes, Perú)

Rostral teeth 6 or 7. Telson lacking proximal, triangular patches of long setae. Thelycum with median plate strongly excavated. Anterior part of sternite XIV with platelike base, and bearing paired, much elongate, tonguelike flaps, extending well beyond midlength of median plate 4

4. Telson with posteriormost pair of lateral spines fixed. Thelycum with platelike base of anterior part of sternite XIV lacking setae on each side, its lateral margins strongly curving posteromesially, forming deep emargination with posterolateral projections (Figure 5A).. ..... *T. fuscina* sp. n.
(Golfo de Tehuantepec and Golfo de Guayaquil)

Telson with posteriormost pair of lateral spines movable. Thelycum with platelike base of anterior part of sternite XIV setose, its lateral margins almost straight, forming about 90° angles with posterolateral projections (Figure 5B) ..... *T. faoea* Loesch and Avila
(From Guatemala to Golfo de Guayaquil)

ACKNOWLEDGMENTS

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ARTIFICIAL RIPENING OF MAATJES-CURED HERRING WITH THE AID OF PROTEOLYTIC ENZYME PREPARATIONS

T. M. RITSKES[1]

ABSTRACT

For manufacturing maatjes-cured herring, the herring caught in the North Sea or Irish Sea is suitable only during months when the proteolytic activity of the appendices pyloricae is sufficiently high. For instance, only North Sea herring caught from May to July has sufficient proteolytic activity for the manufacture of a well-ripened product. For herring caught in other areas, this period may differ considerably. The present work shows that it is possible to use herring caught in other seasons if protease preparations are added to the fish together with salt. The herrings were examined organoleptically, whereas the brines were examined by chemical analysis and by chromatography on Sephadex G-25. No significant differences were found between naturally ripened herring and herring cured by the aid of enzyme preparations. The lipase content of the preparation should be low enough in order to avoid the formation of a fatty acid taste in the cured fish.

Maatjes-type cured herring, the fish product that has been made for centuries aboard Dutch fishing vessels, is usually made from North Sea herring (*Clupea harengus* L.), and in latter years from Irish Sea herring as well.

After being caught, the herring is gibbed and salted promptly. "Gibbing" means the removal of some of the intestines through an incision below the left gill. It is essential that, at this procedure, the appendices pyloricae are left in the fish; according to Luijpen (1959) this organ plays an important role in the formation of the characteristic organoleptic properties of the product. The particular taste and the soft consistency are due to the action of proteolytic enzymes from the appendices pyloricae on the fish flesh.

Since the content of proteolytic enzymes in the fish varies with the seasons, the fish is suitable for the manufacture of maatjes-cured herring only for a few months. Other variables like age and size of the fish or its source may also influence the protease content and in this way restrict the suitability of herring for the maatjes-curing process. For these reasons, there is a demand for a manufacturing method which is less dependent upon the protease activity of the appendices pyloricae.

The aim of our investigation was, therefore, to find the conditions for obtaining an acceptable maatjes-cured herring by the addition of protease preparations. In this way a product with the desired organoleptic properties might be manufactured from herring which have relatively inactive appendices pyloricae and which consequently cannot ripen in a natural way.

MATERIALS AND METHODS

Artificially ripened herring was made by adding protease preparations and salt to gutted fresh or frozen herring. The enzyme preparations were mixed with the salt before salting the herring in the usual way, viz. mixing the herring with a certain quantity of dry salt. Shortly after the addition of salt, a brine is formed which covers the fish entirely. After storage from 7 to 31 days the fish was examined organoleptically and the brines chemically. In most cases the enzyme-treated herring was compared with naturally ripened herring and with eviscerated ("gutted") herring to which no enzyme preparations were added.

The purpose of the chemical analyses was to gather information about possible differences

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between the natural and the artificial ripening process. These analyses included: assay of total soluble nitrogen by the Kjeldahl method, protein determination by the biuret method, and determination of amino nitrogen (as a rough measure for the amino acid content). Assuming that the soluble matter is evenly distributed between the fish and the brine formed, most of the analyses were carried out in the latter.

Some of our experiments are described below. Other experiments gave similar results and are omitted here for ease of survey.

NATURAL RIPENING PROCESS

Frozen maatjes herring that had been caught in the North Sea at the end of May were used. The protein content was 16.4% and the fat content 13.8%. Herring in this stage is normally used for the preparation of maatjes-cured herring.

The protein content was determined by the Kjeldahl method; for the fat determination, the Bligh and Dyer (1959) method was used in a modification according to Ederzeel and Ritskes (1966).

Part of the herring was gibbed and part was gutted. One part of salt was added to 20 parts of herring (light salting). The fish was kept at 3° C for 1 week.

In order to remove proteins which are assumed to be of less importance in the study of the ripening process, the brines were heated for about 15 min at 80° C and then filtered warm over a fluted filter paper. All analyses were carried out with these clarified brines. In this experiment, these analyses included a Kjeldahl nitrogen determination, a measurement of the biuret value according to Strickland, Freeman, and Gurule (1961) and a determination of the amino nitrogen content according to the method of the Dutch Food Law (1963). This latter method is based upon two alkalimetric titrations to different end points. The salt content in the brines was determined by the Volhard method.

In order to elucidate the protein breakdown process during ripening, gel chromatography of the brine was applied; 0.8 ml of a clarified brine was chromatographed on a Sephadex G-25 column[2] (length 44 cm, diameter 1.44 cm; fraction volume 3.4 ml, elution rate 19.8 ml/h), distilled water being used as an eluant. In each fraction the biuret value and the absorbence at 280 nm (nanometers or millimicrons) were measured. The results of the organoleptic evaluations and the chemical analyses are shown in Table 1. The results of gel chromatography are plotted in Figures 1 and 2. As could be expected, the values found in the brine of the gibbed herring were considerably higher than in that of the gutted herring.

TESTING OF TWO ENZYME PREPARATIONS

Frozen spawning herring, caught at the end of August in the North Sea were used. The protein content was 17.6% and fat content 18.2%.

Two enzyme preparations, Pr 8 and TG 21 63, were tested. Their proteolytic activities were determined according to Anson (1938), with denatured hemoglobin as a substrate. Lipolytic activity was determined on olive oil according to Marchis-Mouren, Sarda, and Desnuelle

[2] Reference to trade names in this publication does not imply endorsement of commercial products by the National Marine Fisheries Service.

TABLE 1.—Results of organoleptic evaluation of naturally ripened maatjes-cured herring and of analyses of brine in which herring ripened.

Variation	Organoleptic evaluation of the herring		Analyses of the brine[1]		
	Flavor	Texture	Biuret value (Extinction × 1000 × dilution factor)	Total N (mg N/ml)	Amino N (% w/v)
Gutted	Nontypical	Moderately firm	900	4.4	0.133
Gibbed	Good maatjes-flavor	Optimal (soft)	1720	6.9	0.255

[1] Salt content of brine was 10.5 ± 0.5% w/v.

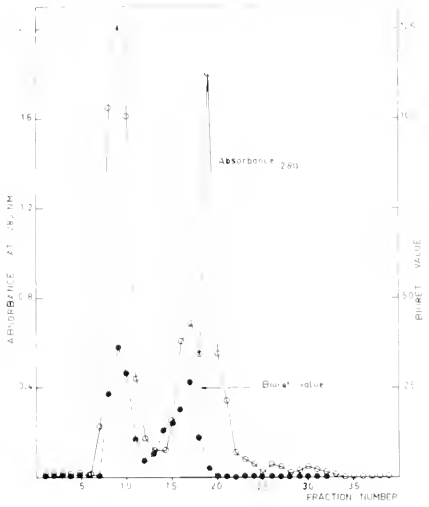

FIGURE 1.—Chromatogram of the clarified brine of gutted maatjes herring on Sephadex when herring were ripened naturally.

(1959). Pr 8 had a proteolytic activity of 5.3 × 10^{-4} U mg and a lipolytic activity of 0.04 Desnuelle units mg; for TG 21 63, these values were 9.1 × 10^{-4} and 18.7, respectively. These preparations, like all others used in this study, were supplied by N. V. Organon, Oss, the Netherlands. Details concerning these preparations are summarized in Table 2.

The proteolytic activity of the preparations was found by measuring the caseinolytic activity at pH 7.5 and 35° C (Ruyssen, 1969). The NF-pancreatin reference standard was used (see National Formulary XIII, 1970, p. 514). The potency of the protease preparations is expressed in terms of the minimum activity required by NF XIII. The lipase activity was measured by potentiometric titration of fatty acids hydrolyzed in an olive oil emulsion at pH 8.0 (Marchis-Mouren, Sarda, and Desnuelle, 1959). The International F.I.P. standard for pancreas lipase was used as a standard (Ruyssen, 1969). One unit forms 1 μmol fatty acid per minute under the conditions of the assay.

In order to eliminate the effect of the intestinal enzymes, the fish was gutted before the enzyme preparations were added.

One part of salt was added to seven parts of herring (w w). The fish was kept at 15° C for 3 weeks. Analyses were taken after 1, 2, and 3 weeks; organoleptic evaluation took place after 2 weeks.

Four variations were tested: (a) gutted; (b) gibbed; (c) gutted and 2.0 g of Pr 8 per kg herring added; (d) gutted and 1.2 g of TG 21 63 per kg herring added.

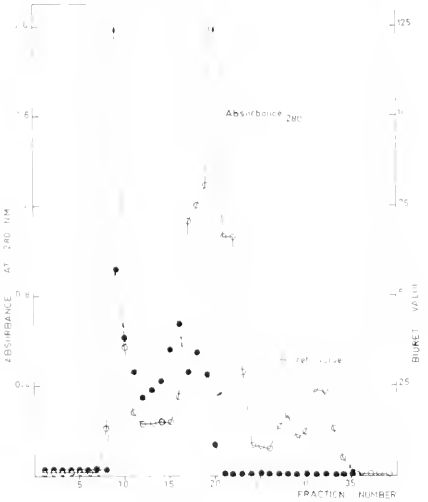

FIGURE 2.—Chromatogram of the clarified brine of gibbed maatjes herring on Sephadex when herring were ripened naturally.

TABLE 2.—Enzyme preparations used in the experiments.

Code No.	Origin and description
TG 21/63	Porcine pancreas powder
Pr 8	Porcine pancreas protease mixture with low lipase activity
Pr 34	Pancreas protease from sheep
Pr 35	Beef pancreas protease mixture
Pr 11/66	Same quality as Pr 8
W	Beef pancreas protease concentrate
CH 32/67 A	Same quality as Pr 8

TABLE 3.—Results of organoleptic evaluations and chemical analyses from experiment using two enzyme preparations.

Variation	Organoleptic evaluation		Analyses of the brine[1]					
			Biuret value			Total nitrogen		
	Flavor	Texture	After 1 week	2 weeks	3 weeks	After 1 week	2 weeks	3 weeks
Gutted	Salty; not ripened	firm	856	1,105	1,305	5.1	6.0	6.0
Gibbed	Not fully ripened	firm	--	1,320	--	--	8.1	--
Gutted + Pr 8	Ripened flavor	soft	2,620	3,670	4,320	10.0	11.8	13.4
Gutted + TG 21/63	Ripened flavor but with an unpleasant fatty acid flavor	soft	2,740	3,950	4,680	10.4	13.1	15.0

[1] The salt content in the brine was 20 ± 2% w/v.

The brine was analyzed as described earlier, but with the omission of the amino-N determination.

The results of the organoleptic evaluations and the chemical analyses are shown in Table 3. They show that, by the addition of enzyme preparations to gutted herring, it is possible to obtain a product with "maatjes-cured" organoleptic properties. A high lipolytic activity, however, seems to be undesirable.

Both the biuret value and the total nitrogen content in the brine increase during the ripening period. There seems to be a relation between the values found and the degree of ripening.

ARTIFICIAL RIPENING OF FRESH SPENT HERRING WITH ENZYME PREPARATIONS LOW IN LIPASE ACTIVITY

After the herring has spawned, the uptake of food steps and the fat content decreases gradually to relatively low values, e.g., 5 to 8%. Since the proteolytic activity of the appendices pyloricae is low in this period, the fish as such is unsuitable for the manufacture of maatjes-cured herring.

Fresh, spent herring, caught in February in the Irish Sea were used. The protein content was 17.1% and the fat content 5.2%.

Activity of the enzyme preparations tested is shown in Table 4.

In view of the results obtained in the experiment on spawning herring, the proteolytic activity in the artificial maatjes-cured herring was reduced in this experiment.

Six variations were tested: (a) gutted; (b) gibbed; (c, d, e, f) gutted and added respectively: 1.0 g Pr 34, 1.0 g Pr 35, 1.0 g Pr 11/66 and 55 mg W per kg of fish. The fish was kept at 3° C for 3 weeks. One part of salt was added to 10 parts of herring.

The brine was analyzed as described earlier. In analyses of the herring, 30 g of ground herring fillets were homogenized with 100 ml of water in an Ultra-Turrax mixer. The mixture was heated to 80° C and, after cooling to room temperature, filtered through fluted filter paper. In the extract thus obtained, the same analyses as earlier described for the brine were carried out. Gel chromatography was performed as described earlier.

The results of the organoleptic evaluations and the chemical analyses are shown in Table 5.

The brines of the variations "gutted" and "gutted + Pr 35" were chromatographed. The results are plotted in Figures 3 and 4.

With the preparations Pr 35 and Pr 11/66, an acceptable product was obtained. W gave rise to a good texture but developed less flavor. Pr 34 caused some off-flavor, probably because of the comparatively high lipolytic activity. It seems to us that a lipolytic activity lower than 1 U mg is desirable for a preparation which has a protease activity equivalent to the minimum activity required by NF XIII.

TABLE 4.—Proteolytic and lipase activities of enzyme preparations.

Enzyme preparation	Proteolytic activity according to NF XIII	Lipase activity: Desnuelle U/mg
Pr 34	1.6 × NF	1.8
Pr 35	1.0 × NF	0.2
Pr 11/66	3.0 × NF	0.2
W	20 × NF	0.6

TABLE 5.—Results of organoleptic evaluations and chemical analyses from experiment with the artificial ripening of fresh spent herring with enzyme preparations low in lipase activity.

Variation	Organoleptic evaluation		Chemical analyses					
			In the brines[1]			In the extracts		
	Flavor	Texture	Biuret value	Total N	Amino N	Biuret value	Total N	Amino N
Gutted	Nontypical	Moderately firm	1,345	5.6	0.138	192	0.55	0.017
Gibbed	Nontypical	Moderately firm	1,665	7.8	0.178	--	--	--
Gutted + Pr 34	Fair, but with some off-flavor	Soft	2,690	8.8	0.255	--	--	--
Gutted + Pr 35	Fair	Too soft	2,900	9.1	0.252	--	--	--
Gutted + Pr 11/66	Fair	Soft	2,355	7.3	0.210	487	1.29	0.036
Gutted + W	Nontypical	Soft	2,460	8.2	0.224	--	--	--

[1] The salt content in the brines was 12 ± 1% w/v.

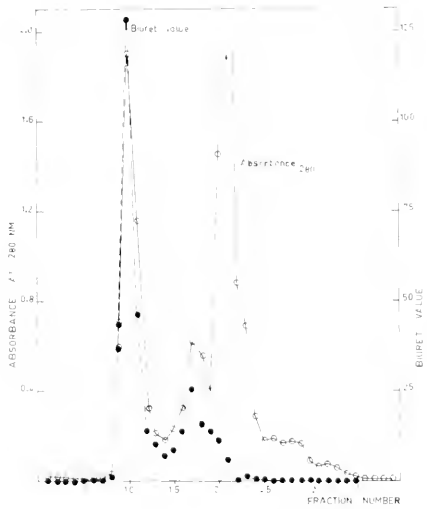

FIGURE 3.—Chromatogram of the clarified brine of gutted herring, without the addition of enzymes on Sephadex G-25, in experiment on artificial ripening of fresh spent herring with enzyme preparations low in lipase activity.

There exists a relation between the texture of the herring and the biuret value found in the brine. Between texture and total amount of nitrogen in the brine no apparent relation was shown. The ratio total N to amino N is about the same in the brine and in the herring extract, indicating that analysis of the brine give information about the ripening process in the herring.

The chromatograms show that, in the brine of the ripened herring, the amount of biuret-positive material with larger retention times has increased. This indicates that the amount of small protein fragments increased.

The chromatographic pattern of the brine of an artificially ripened herring (Figure 4) does

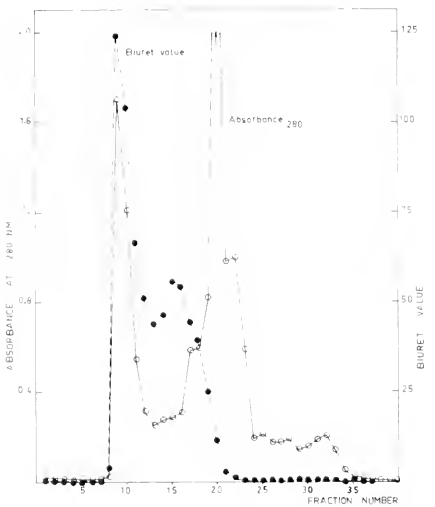

FIGURE 4.—Chromatogram of the clarified brine of gutted herring, with the addition of the enzyme Pr 35, on Sephadex G-25, in experiment on artificial ripening of fresh spent herring with enzyme preparations low in lipase activity.

not differ essentially from that of the gibbed herring brine (Figure 2). With the methods used, no obvious difference was found between the natural and the artificial ripening process.

THE ARTIFICIAL RIPENING OF LEAN HERRING AND ITS RELATION TO CHANGES IN THE RESULTS OF BRINE ANALYSIS

Fresh herring caught in the North Sea at the beginning of April were used. The protein content was 16.7%; fat content, 10.6%.

Two variations were studied: (a) gutted; (b) gutted and with 1.0 g of Pr 35 per kg of herring added. One part of salt was added to 10 parts of herring. The fish was kept at 3° C. Samples for analysis were taken after 11, 17, 24, and 31 days. Organoleptic evaluation was carried out after 24 days.

The brine was analyzed as described earlier.

After 24 days at 3° C, organoleptic evaluation showed: variation (b) had a soft texture and a well-ripened flavor, whereas variation (a) was still firm and had less flavor.

Results of chemical analysis of the brine are plotted in Figure 5 and summarized in Table 6. The salt content in the brines was 16 ± 2% w/v.

During the ripening process there is a steady increase in biuret value, total N, and amino N in the brine. This finding demonstrates again the relation between these values and the degree of ripening.

THE ARTIFICIAL RIPENING OF FRESH LEAN HERRING WITH DIFFERENT AMOUNTS OF A PROTEASE PREPARATION

Fresh spent herring caught near the Hebrides in August was used. This herring contained 17.4% of protein and 8.2% of fat.

In this experiment an enzyme preparation CH 32 67 A was tested, with a proteolytic activity of 1.93 times NF XIII units and a lipolytic activity of 0.3 Desnuelle units per mg.

One part of salt was added to 10 parts of fish. Six variations were tested: (a) gutted; (b) gibbed; (c, d, e, f) gutted and 0.5, 1.0, 2.0, and 5.0 g of CH 32 67 A added, respectively. The fish was kept at 3° C for a month, then evaluated and analyzed.

The brine was analyzed as discussed earlier. Results are shown in Table 7.

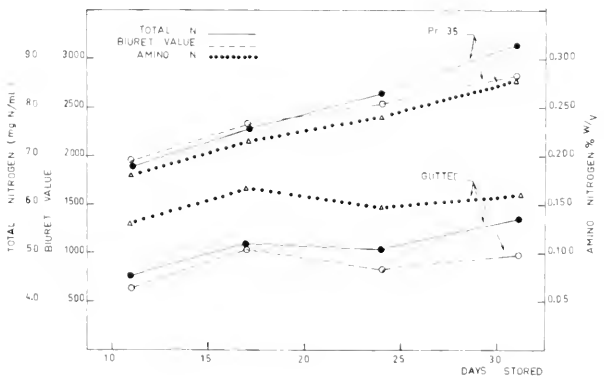

FIGURE 5.—Results of analyses of clarified brines of gutted herring with and without addition of Pr 35, in experiment on the artificial ripening of lean herring and its relation to changes in the result of brine analysis.

TABLE 6.—Chemical analyses of the brines[1] in experiment on artificial ripening of lean herring.

Brines	Gutted	Gutted + Pr 35
Biuret value:		
after 11 days	625	1,915
after 17 days	1,010	2,320
after 24 days	840	2,520
after 31 days	960	2,820
Total N:		
after 11 days	4.5	6.8
after 17 days	5.2	7.6
after 24 days	5.1	8.3
after 31 days	5.7	9.3
Amino N:		
after 11 days	0.131	0.183
after 17 days	0.165	0.215
after 24 days	0.146	0.238
after 31 days	0.160	0.282

[1] The salt content in the brines was 16 ± 2% w/v.

An addition of 0.5 g of CH 32/67 A per kg of herring is too low to obtain a well-ripened product under these conditions. An addition of 1.0 g per kg, however, seems somewhat too high. A suitable dose is probably the amount of protease that corresponds with a proteolytic activity of 2000 mg-eq NF XIII powder per kg of herring.

In the brines (b) and (d) the same amino-N contents were found, but the biuret value in (d) was considerably higher than in (b). This suggests that the action of the enzyme preparation is particularly the breakdown of muscle protein to larger fragments, whereas the endogenous enzymes show more peptidase activity. This is in accordance with the observation that an overdose of the enzyme preparation results in a very soft rather than in a strong-tasting herring.

SUMMARY

The addition of a certain quantity of a protease preparation to herring which is unsuitable for maatjes curing has a favorable effect on both flavor and texture of the herring.

An addition of 2000 mg-eq NF XIII powder per kg of herring is proposed. Doubling or halving this amount had a pronounced effect on the organoleptic properties of the cured herring. The lipase activity should be low.

In the brines the biuret value, the total N content and the amount of amino N increase gradually. There appears to be a distinct relation between the biuret value in the clarified brine and the consistency of the herring; the same is true for the amino N content in the brine and the flavor of the herring.

The ratio biuret value: amino N content found in the brine was higher for artificially ripened herring than for the naturally ripened product. This finding indicates that the enzyme preparations are poorer in peptidase activity than the appendices pyloricae from the herring.

The protein breakdown in the artificially ripened herring, however, does not seem to be essentially different from that in the naturally ripened herring. The patterns obtained by chromatography of the brines over Sephadex G-25 did not show any essential difference.

ACKNOWLEDGMENTS

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TABLE 7.—Results of organoleptic evaluations and chemical analyses from experiment on artificial ripening of fresh lean herring with different amounts of a protease preparation.

Variation	Organoleptic evaluation		Chemical analyses of brines[1]		
	Flavor	Texture	Biuret value	Total N	Amino N
Gutted	Salty; no ripened flavor	Firm	1,300	5.4	0.195
Gibbed	Nontypical	Moderately firm	1,640	9.0	0.328
Gutted + 0.05% CH 32/67 A	Fair	Moderately soft	2,320	8.0	0.274
Gutted + 0.10% "	Fair	Optimal (soft)	2,875	9.2	0.334
Gutted + 0.20% "	Strongly ripened	Too soft	3,210	10.7	0.398
Gutted + 0.50% "	Not tasted	Too soft	3,650	11.9	0.465

[1] The salt content in the brines was 12 ± 1% w/v.

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LABORATORY STUDIES OF PREDATION BY MARINE COPEPODS ON FISH LARVAE

KURT LILLELUND[1, 2] AND REUBEN LASKER[3]

ABSTRACT

A variety of marine copepods have been shown to fatally injure or capture and ingest young anchovy larvae in the laboratory. *Labidocera jollae*, *L. trispinosa*, and *Pontellopsis occidentalis* (family Pontellidae), species common to surface waters of the California Current, are effective predators of larval fish. The copepods can be attracted by the vibrations of the larval tail beat and react by biting or capturing the fish larvae. Cruising speeds for these copepods varies from 1.5 to 4 body lengths per second which, coupled with continuous swimming behavior, results in extensive searching by the copepod for prey. In laboratory experiments, when the ratio of larval fish prey to *L. jollae* female individuals was low (<10:1 in 3500 ml), all of the larvae were killed in 24 hr where "killing" refers to both capture-ingestion and biting resulting in a fatality. If the ratio was higher, killing increased but rarely reached 100% mortality of the larvae. *L. trispinosa* males and females never killed all the larvae offered to them in 24 hr in 3500 ml although more larvae were killed as the number offered was increased. Increased swimming and escape ability developed as anchovy larvae became older and were not caught or bitten as effectively as younger ones by copepods. However, killing of larvae by *P. occidentalis* was unaffected by the age of the larvae up to 3.5 days old.

When *Artemia salina* nauplii were substituted for larval fish as prey for *L. trispinosa*, the amount of grazing was proportional to the ratio of nauplii to copepods. If the number of *Artemia* nauplii was less than 11-14/liter per copepod in 3500 ml all the nauplii were killed in 24 hr. When the density of nauplii was increased, more were killed but never all of them. In experiments where nauplii and yolk-sac larvae were offered together as prey the mortality of the larvae due to predation declined in proportion to the increase in the number of nauplii provided.

Caloric requirements were calculated from oxygen consumption measurements and showed that only 1 to 4 anchovy larvae are required per day per copepod to satisfy the metabolic needs of *Labidocera*, depending on the species and the sex. This number is far less than can be killed or captured if the density of larvae is high enough.

In large (140 cm) vertical cylinders larvae and *L. trispinosa* were distributed within 25 cm of the surface in the dark. Data are presented which show that *L. trispinosa* and anchovy larvae also co-occur in the upper few meters under the surface of the sea. This is probably also true for *P. occidentalis* and *L. jollae*. No data are yet available on the relative density of predatory copepods and fish larvae where they co-occur or their possible predator-prey interactions in the sea.

Huge mortalities of larval fish are known to occur in the sea. From the time of Hjort (1914) these have been attributed mainly to the lack of the proper food when the larvae begin to feed (see review by Blaxter, 1969). Undoubtedly other biotic and abiotic factors are also involved in larval fish mortality, but comparatively little work has been done to measure their effect. Among the possible causes of larval-fish mortality, predation by other zooplankters may be an important factor. Freshwater aquarists have known for some time that copepods must be eliminated from fish rearing tanks or high mortalities of young larvae or fry will occur (Davis, 1959). Lillelund (1967) reviewed the literature pertaining to predation by freshwater copepods on fish larvae and described the predatory behavior of cyclopoid copepods as he observed them

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in the laboratory. Zooplankters in general and copepods in particular have been seen to devour marine fish larvae. For example, in an early study on the rearing of marine fish, Garstang (1900) noted that the harpacticoid copepod *Idya furcata* (= *Tisbe furcata*) was a larval-fish predator. Subsequently Lebour (1925) from her examination of living marine plankton, concluded that a variety of zooplankters eat fish larvae and noted that "many jellyfishes and *Pleurobrachia* besides *Sagitta* and *Tomopteris* will readily eat small fishes." Lebour also illustrated the capture of an anglerfish larva (*Lophius piscatorius*) by the copepod *Anomalocera pattersoni* (family Pontellidae).

Despite this information on the predatory behavior of copepods, there is virtually no behavioral information available on the ability of marine copepods to capture and ingest or fatally injure fish larvae, although many marine copepods are known to be carnivorous (Gauld, 1966) and other incidental reports of copepods predatory on marine fish larvae have been made (Wickstead, 1965; Petipa, 1965). Furthermore, the possible importance of predation on fish larvae as it affects the determination of year class strength through larval fish mortality has been generally ignored, probably because of the lack of pertinent quantitative laboratory and field information.

In this study we present the results of experiments designed to measure quantitatively the ability of three pontellid marine copepods, *Labidocera trispinosa*, *L. jollae*, and *Pontellopsis occidentalis*, to capture or fatally injure larvae of the northern anchovy, *Engraulis mordax*, an important commercial fish of the California Current. The behavior of copepods and larvae which bears on the susceptibility of the latter to predation is also described in detail.

METHODS

Copepods and anchovy eggs were captured with a 0.5-m-mouth-diameter plankton net (0.333-mm mesh) towed at the surface in coastal water off San Diego, Calif., between March and August 1970. The copepods were separated from other plankton with a large bore pipette on shipboard, diluted with surface water in liter jars, and kept at sea water temperature (about 15° C) in an insulated chest until returned to the laboratory.

All experiments were performed in 3500-ml beakers in the dark because *Labidocera* were phototactic and attracted to the light source and *Pontellopsis* was inhibited in its attacks on larvae in the light. In an earlier study on freshwater cyclopoid copepods, Lillelund (1967) used a constant level, continuous flow device which we used also for maintaining marine copepods in good condition in the laboratory (Figure 1). However, it was more convenient to do all predation experiments in static water over a 20- to 24-hr period, since the copepods we investigated swim continuously throughout the small volume (3500 ml), obviating the need for continuously circulated water. A constant temperature of 18° C was maintained in the beakers by placing them in a running seawater bath.

Mortality of the larvae was measured by taking the difference between the number of larvae at the beginning of the experiment and those remaining alive at the end. Some mortality not associated with predation always occurred, hence control vessels containing larvae alone were always provided and the results of experiments corrected for larvae dead of other causes. In all experiments, this natural mortality never exceeded 10%.

Anchovy eggs were sorted in the laboratory according to their stage of development and newly hatched larvae were used as prey in the predation experiments. When older larvae were required they were reared according to the method of Lasker et al. (1970), except that the rotifer *Brachionus plicatilis* was substituted for snail veligers as larval-fish food (Theilacker and McMaster, in press).

Oxygen consumption measurements were made by Warburg manometry. Usually 18 to 22 copepods were put into 3 ml of seawater in a Warburg flask and oxygen uptake monitored for 8 hr at 18° C. Dry weight of individual copepods was measured with an electrobalance to ± 2 μg.

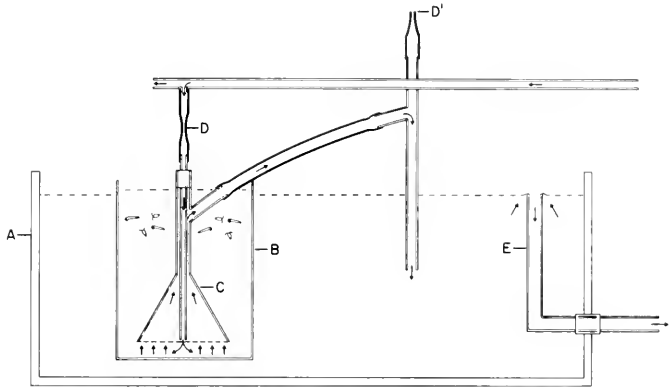

FIGURE 1.—Constant level device for maintaining copepods and fish larvae. The temperature of the 3500-ml beaker (B) was kept constant with running seawater in a water table (A) whose capacity was 610 liters. Plankton netting (0.333-mm mesh) was held over the mouth of the 500-ml funnel (C) with a section of bicycle tire tube. Rubber tubing showing constrictions as D and D' had screw-type clamps to regulate flow. E is the drain. Note that the level of seawater is higher in the beaker than in the bath. The drawing is not to scale.

MARINE COPEPODS CAPABLE OF KILLING ANCHOVY LARVAE

Before choosing *Labidocera* and *Pontellopsis* for experimental work we tested a number of copepod species for their ability to capture or fatally injure newly hatched yolk-sac larvae of the northern anchovy. For each test, five fish larvae were isolated in 200 ml of seawater in a Petri dish at room temperature (20° C) usually with two or three copepods of a particular species to be tested. Of the local copepod species observed, the following fatally injured anchovy larvae by biting them or captured and ingested them:

Acartia dana and *A. tonsa*
Euchirella rostrata and *E.* sp.
Labidocera jollae and *L. trispinosa*
Pleuromamma borcalis
Pontellopsis occidentalis
Euchaeta acuta
Euaetideus acutus
Candacia bipinnata

Because the two species of *Labidocera* listed above are common to waters adjacent to San Diego and were readily available, most of our experimental work was done with them. When *Pontellopsis occidentalis* became abundant, we also collected some information about its predatory behavior relative to fish larvae.

Although capture and ingestion of fish larvae was commonplace under laboratory conditions, it is rare to find a copepod with a captured fish larva in Formalin-preserved plankton. This may be the result of the Formalin preservation common on shipboard which, we have observed, usually causes copepods to drop larvae.[4]

[4] We have found that if a copepod has captured a larva it will retain the larva if both are transferred together to a slight melted depression in an ice cube with a pipette and preserved with a drop of 3% Formalin.

SWIMMING AND FEEDING BEHAVIOR OF *Labidocera*

REACTION TO THE LARVAL TAIL BEAT; BITING AND INGESTION OF LARVAE

We noted that individual *Labidocera* ignored motionless fish larvae or floating eggs. However, when a larva beat its tail in the close vicinity of a swimming labidoceran, the copepod swam immediately toward the beating tail and grasped the larva. The tail beat of the larva was often stimulated by the chance touch of a copepod's antenna. Figures 2a and 2b show a *L. jollae* female which caught a 3-day-old anchovy by the tail and partially ingested it. Figure 3 shows another larva caught behind the head by another female. Response to the larval tail beat is typical of all the copepods we have observed which attack fish larvae.

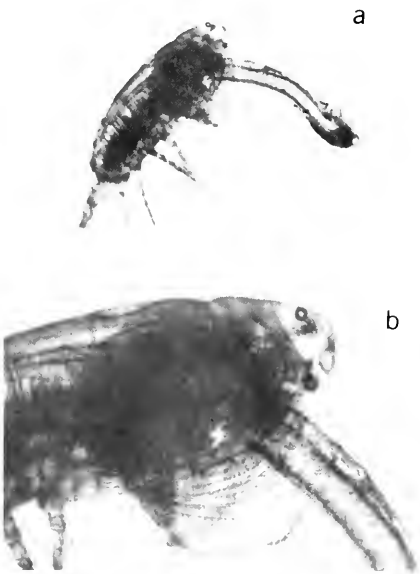

FIGURE 2.—(a) *Labidocera jollae* female, 3 mm long, and a 3-day-old anchovy larva 6 mm long which was captured by the tail and half ingested. (b) Enlargement of the head and setae of the copepod shown in 2a.

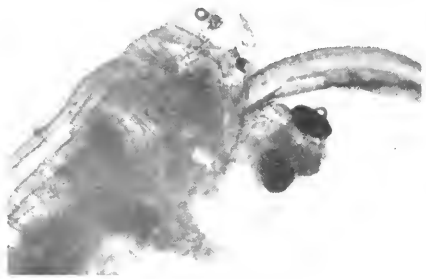

FIGURE 3.—The head of a *Labidocera jollae* female showing a newly captured 3-day-old anchovy larva caught behind the head.

Often a copepod would capture, then drop a larva, inflicting a wound by biting the thin epithelium. The anchovy larval skin is only about 2 to 3 μ thick in the finfold and tail regions and appears to be easily injured. In every instance, a bite which damaged the larval skin resulted in the death of the larva. Therefore, in our experiments mortality due to a copepod was the result of either actual capture and ingestion of a larva or biting that resulted in damage fatal to the larva. Hence, the number of larvae reported as "killed" in an experiment is the sum of fatalities due to biting and the number of larvae actually ingested. For both sexes of each *Labidocera* species the time of ingestion of anchovy larvae varied between 6 and 25 min. In one instance an *L. trispinosa* male caught and completely consumed two larvae in 1 hr. If we increased the number of larvae to six or more in a 200-ml Petri dish containing two copepods, mortality through biting alone increased and the time during which a larva was held by a copepod varied from a few seconds to minutes. For example, an *L. jollae* female attacked six larvae in 50 min. The individual larvae were held only 10 to 60 sec and not ingested. All six larvae died subsequent to the attack.

POSITIVE PHOTOTAXIS AND SWIMMING SPEED

Labidocera jollae and *L. trispinosa* are positively phototactic, and when confined to a beaker of seawater illuminated from above, concentrate at the water surface-beaker interface which is the brightest area. Copepods were induced to swim in the main body of water by wrapping the beaker with black paper with 1 cm lapped around the rim. This effectively eliminated the bright area and resulted in random swimming movements of the copepods near the surface.

Swimming distances of copepods were traced for 3 min in two dimensions on a clear acetate sheet laid over a glass plate on top of a 3.5-liter beaker. The distances were measured with a map measurer. Vertical movements were very slight, thus negligible, in these experiments because of the highly phototactic behavior of the individuals. Labidocerans can swim continually over relatively large areas in short periods of time (Vlymen, 1970). Comparative speeds for individuals are shown in Figure 4; on the average a *L. jollae* female swims 62 cm/min (3-4 body lengths/sec) and the male swims 22 cm/min (1.5-2 body lengths/sec). Both sexes of *L. trispinosa* swim 33 to 37 cm/min (2-3 body lengths/sec). Although *L. jollae* females swim in a seemingly random pattern, the males usually swim in straight lines for a few seconds then swim in circles and cover a small area intensively.

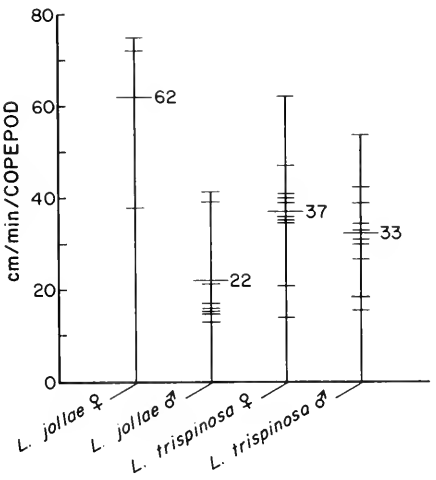

FIGURE 4.—Swimming speeds for individuals of *Labidocera*. Each small horizontal bar represents the speed of one animal; the large bar is the mean speed.

KILLING EFFICIENCY OF *Labidocera*

We discovered that if the ratio of anchovy larvae to *L. jollae* females was low (<10:1), all or almost all the larvae in 3500 ml would be killed within 20 to 24 hr in the dark. Two experiments were done which illustrate this. In the first, 30 anchovy larvae were confined with a variable number of *L. jollae* females (Figure 5) resulting in concentrations of larvae to copepods

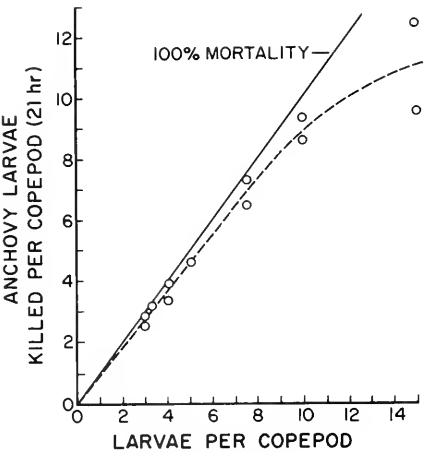

FIGURE 5.—Mortality of *Engraulis mordax* larvae, 0 to 1 day old, resulting from predation by different numbers of *Labidocera jollae* females. In each experiment 30 larvae were presented to 2 or more copepods in 3500 ml for 21 hr. Thus where 3 larvae per copepod is indicated on the abscissa, 30 larvae and 10 copepods were used; at the other extreme 15 larvae per copepod indicates 30 larvae and 2 copepods. The unbroken line is the theoretical 100% larval mortality curve.

of 3:1 to 15:1. In the other, only single *L. jollae* females were tested and the number of larvae varied to provide ratios of larvae to copepods of 5:1 to 40:1 (Figure 6). The results were

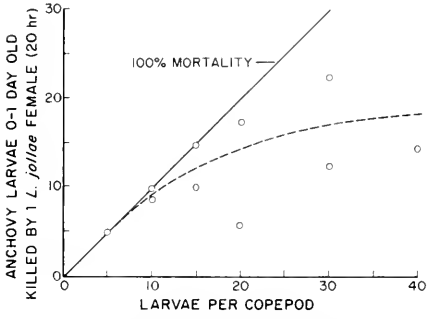

FIGURE 6.—Mortality of *Engraulis mordax* larvae, 0 to 1 day old, resulting from predation by single *Labidocera jollae* females. In each experiment 1 copepod was confined with 5 to 40 larvae in 3500 ml for 20 hr. The unbroken line is the theoretical 100% larval mortality curve.

similar in the two experiments; when the ratio was approximately 10:1 or less, it was usual for all larvae to be killed. When more than 10 larvae were available per copepod, more larvae were killed per copepod but the mortality dropped below 100%. In similar experiments two *L. trispinosa* females were tested with larvae to copepod ratios varying from 2:1 to 15:1. *L. trispinosa* females were much less efficient than *L. jollae* females and never killed all the larvae presented to them in 24 hr (Figure 7). Males of both species were similar in killing efficiency to *L. trispinosa* females. Based on these results, further predation experiments were performed over 20 to 24 hr using 30 larvae with two *L. jollae* females or five males; experiments performed with either sex of *L. trispinosa* had 30 larvae and 5 copepods. The comparative predatory ability of labidocerans is shown graphically in Figure 8. The mean number of anchovy larvae killed by *L. jollae* females was 15. *L. trispinosa* males and females had mean kills of 1 and 2

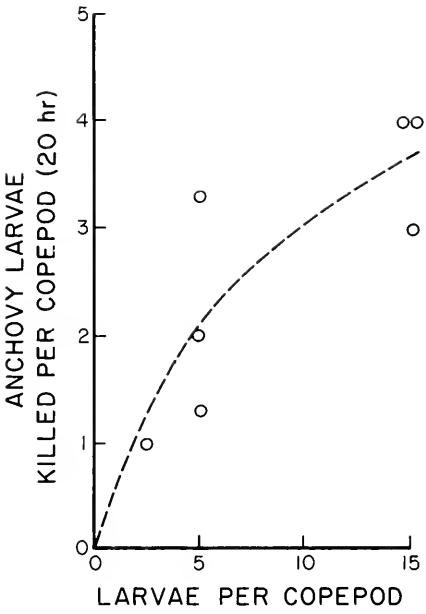

FIGURE 7.—The effect of increasing the density of larval anchovies on the predatory behavior of *Labidocera trispinosa* females. Each dot represents a separate experiment with 2 or 3 copepods and from 5 to 45 larvae.

larvae respectively in 24 hr, markedly less than *L. jollae* females. The high killing rate by *L. jollae* females reflects the longer distances and greater volume covered by them owing to their larger size. *L. jollae* females are approximately 0.2 mg dry weight, and males, 0.1 mg. Female *L. trispinosa* average about 0.1 mg dry weight; males, 0.09 mg.

EFFECT OF THE AGE OF THE LARVA ON PREDATION BY *Labidocera*

We noted in our experiments that *Labidocera* became less efficient in killing anchovy larvae as the larvae aged. The anchovy larva is 2.5 mm

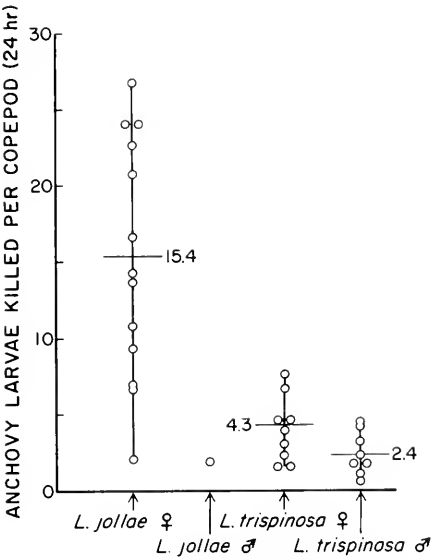

FIGURE 8.—Predation by individual *Labidocera jollae* and *L. trispinosa* under comparable prey density. Experiments were conducted in the dark for 24 hr in 3500 ml. Each circle represents the results of one experiment. The horizontal bar is the mean value for each series of experiments.

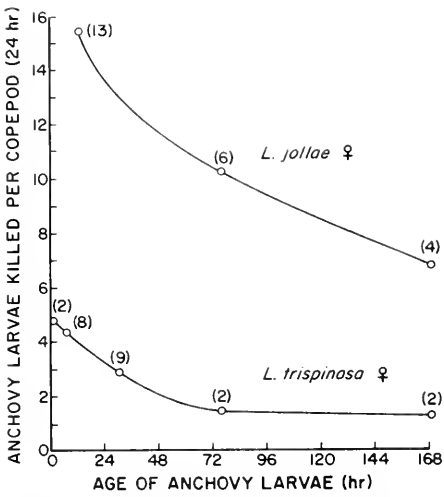

FIGURE 9.—The effect of the age of the anchovy larva on predation by *Labidocera jollae* and *L. trispinosa* females. Each open circle is the mean of the number of experiments shown in parentheses. The age of the larva at the beginning of each experiment is given on the abscissa.

long when it hatches from the egg. Yolk-sac anchovy larvae have unpigmented eyes, lack a mouth and gills, and the yolk is invested with lipid (Bolin, 1936); when newly hatched the anchovy larva is very slightly buoyant. The newly hatched larva remains motionless most of the time at this stage and swims only sporadically. As it develops, swimming activity increases, occurring about 5% of the time at hatching to 25% on the second day and 50% by the third (John R. Hunter, personal communication). Figure 9 indicates a rapid decline in predation in the dark by *L. trispinosa* and *L. jollae* females as the larva grows older, presumably as a result of the latter's increased swimming and sensory ability. Effective predation is restricted therefore to yolk-sac larvae.

EFFECT OF LARVAL ANCHOVY DENSITY ON *L. jollae* PREDATION

Given 2 to 3 days, single *L. jollae* females can kill by capture or biting all of 30 young anchovy larvae in 3500 ml. This is shown in a mortality curve (Figure 10) constructed from the results of a series of experiments, each of which had a number of newly hatched larvae (30 or less) at the start confined with a single *L. jollae* female. The density of larvae per unit volume (within the limits of these experiments) seemed to have little or no effect on the kill rate until there was only one larva remaining per 700 ml, when the rate due to predation by the copepod declined drastically. Our experience with predation experiments in 3500 ml volumes suggested that anchovy larvae were randomly distributed in this relatively small volume and that in the dark, at least, each *L. jollae* female could almost

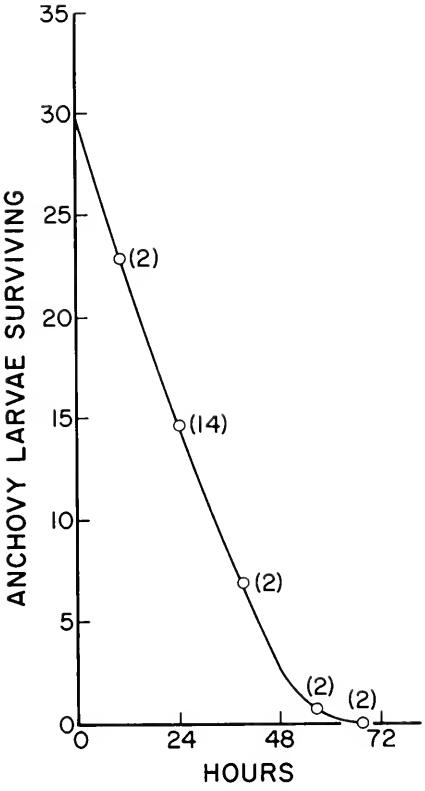

FIGURE 10.—Survival of 0- to 1-day-old anchovy larvae in the presence of a single *Labidocera jollae* female. The curve is a composite from a series of experiments in 3500 ml starting at different larval densities per copepod where the time was noted after the capture of a number of larvae. The numbers in parentheses indicate the number of experiments ending at each point.

completely search half this volume in 24 hr, resulting in continuing random contact with and killing of most, although not all, of the suspended larvae when two or more *L. jollae* females were present.

THE EFFECT OF AN ADDITIONAL PREY ON LARVAL PREDATION BY *Labidocera*

Recent experiments by Brooks (1970) with *Labidocera trispinosa* showed that this copepod selects *Artemia salina* nauplii over copepod nauplii from the plankton. She concluded that *Artemia* nauplii are selectively grazed because they are relatively less mobile, hence more easily captured.

We tested predation by *L. trispinosa* on *Artemia* nauplii and found that grazing corresponded roughly to the results we obtained when fish larvae alone were killed, i.e., up to a certain concentration all *Artemia* nauplii were killed in the experimental container in the dark over 24 hr. Survivors were found only if the number of nauplii exceeded 11-14 nauplii liter^{-1} copepod^{-1}. As the density of nauplii was increased more were killed. This result was the same whether experiments were performed in 3500-ml beakers or in 200-ml Petri dishes (Figure 11).

When *Artemia* nauplii in various concentrations and 30 anchovy larvae were offered together to five *L. jollae* males or five *L. trispinosa* males or females, larval mortality decreased in

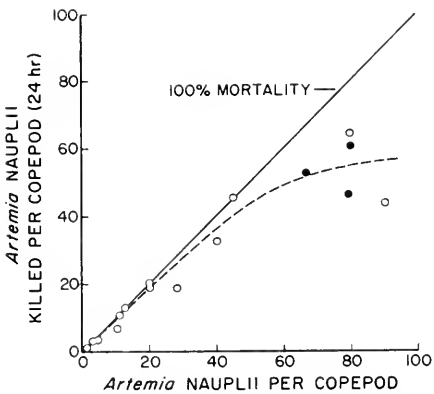

FIGURE 11.—Mortality of *Artemia* nauplii due to *Labidocera trispinosa* female predation at different densities of nauplii per copepod. Closed circles indicate experiments done in 200-ml Petri dishes.

proportion to the number of *Artemia* nauplii present. The results of these experiments are shown in Figure 12. There was a 50% decrease in mortality of larvae with *L. trispinosa* females, *L. jollae* males, and *L. trispinosa* males when the nauplii concentration was approximately 220, 150, and 100 nauplii/liter, respectively. The decrease in larval mortality is accentuated if *Artemia* nauplii are offered to *Labidocera* when older larvae are present. Fewer *Artemia* nauplii need to be present to depress the predation mortality on older larvae (Figure 13). The ease with which *Labidocera* can capture *Artemia* may make it less likely that fish larvae will be attacked. As the larvae age, this effect is compounded since it has become even more difficult to catch larvae and hence relatively less taxing for the copepod to take *Artemia* nauplii.

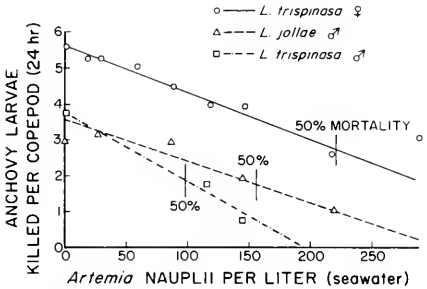

FIGURE 12.—Predation by *Labidocera jollae* males and both sexes of *L. trispinosa* on anchovy larvae when *Artemia* nauplii were also available to the copepods. Fifty percent reduction in larval mortality occurred when *Artemia* nauplii numbered 220/liter with *L. trispinosa* females, approximately 150/liter with *L. jollae* males and 100/liter with *L. trispinosa* males.

CALORIC REQUIREMENT OF *L. jollae* AND *L. trispinosa*

We noted that the vigor of *Labidocera* individuals declined with time if they were not fed or fed only *Artemia* nauplii. For example, after 1 week in the laboratory the activity of copepods fed only *Artemia* was diminished so that a copepod's ability to capture larvae was about one-

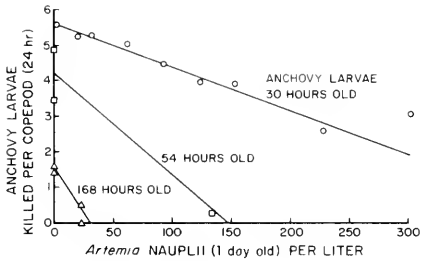

FIGURE 13.—The effect of the age of the anchovy larva and the addition of an extra prey (*Artemia* nauplii) on larval mortality due to predation by *Labidocera trispinosa* females.

half that of a newly caught copepod. *Labidocera* died after 2 or 3 days of starvation. This was preceded by a decrease in swimming activity which was reflected in a lower respiration rate and dry weight of individuals. In Figure 14 we give comparative respiration rates and dry weights for *L. jollae* females (a) newly caught, (b) larval-fish fed, and (c) starved for 2 days. These results show an enhanced respiratory rate for fed and presumably healthier animals and a drastic decline due to short term starvation.

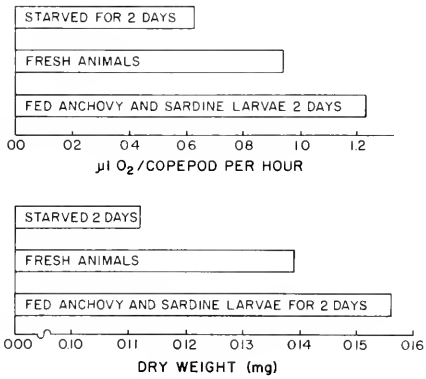

FIGURE 14.—Comparative oxygen consumption and individual dry weight measurements of *Labidocera jollae* females under starved and well-fed conditions at 18° C.

Further comparisons of respiration rates were made only between newly caught and larval-fish fed *L. jollae* females, males, and *L. trispinosa* males and females (Figure 15). In each instance there was an increase in respiratory rate after feeding on anchovy larvae. The caloric requirement of *Labidocera jollae* and *L. trispinosa* was calculated from oxygen consumption data to estimate the number of larvae which could sustain the copepod in a healthy condition at 18° C (Table 1). In spite of *Labidocera's* ability to kill large numbers of fish larvae or *Artemia* in a day, actual caloric requirements may be met by ingestion of only a few (1-4) larvae or nauplii (4-16).

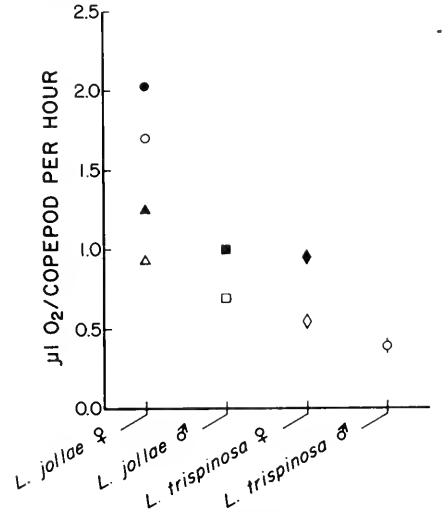

FIGURE 15.—Oxygen consumption by newly caught (open symbols) and larval-fish fed (closed symbols) labidocerans at 18° C.

PREDATION ON FISH LARVAE AND *Labidocera* BY *Pontellopsis occidentalis*

Pontellopsis adults and copepodites stages IV and V can kill by biting or capture and ingestion of anchovy larvae; older larvae (up to 3.5 days old in our test) were killed by this copepod as easily as yolk-sac larvae. Each stage V copepodite killed three larvae per day on the average and each adult female killed about 11 per day (Figure 16). We observed also that *Pontellopsis* attacked and ate *Labidocera* spp. when they were confined to the same beaker.

CO-OCCURRENCES OF PREDATORY COPEPODS AND FISH LARVAE IN THE SEA

In the laboratory we noted that 30 to 40% of the *Labidocera* individuals resided in the upper 5 to 25 cm of 140-cm-deep, 17.5-cm-diameter tanks—both in darkness and in the light. Yolk-sac anchovy larvae occupied a similar stratum because they are slightly buoyant. In the sea, spawning by anchovies occurs mostly in the upper 10 m but occasionally may occur relatively deeply (Ahlstrom, 1959). This prompted us to

TABLE 1.—Oxygen consumption and the calculated number of anchovy larvae required to sustain the respiratory requirements of *Labidocera jollae* and *L. trispinosa* per day at 18° C. The oxycalorific equivalent of 1 µliter of oxygen is 0.005 calorie. Yolk-sac anchovy larvae weigh 0.01 mg dry weight and contain 0.054 calorie. These data assume 100% digestive assimilation and an $RQ = 0.8$ for each copepod. Approximately four *Artemia* nauplii are calorimetrically equivalent to one anchovy larva.

Species and sex	Q O_2 µliter/mg dry weight/hr	O_2 consumption µliter/copepod/hr	Average dry weight per copepod (mg)	Anchovy larvae required/day
Labidocera jollae	11	2.0	0.19	4
L. jollae	11	1.0	0.095	2
L. trispinosa	7.7	0.96	0.12	2
L. trispinosa	4.3	0.40	0.092	1

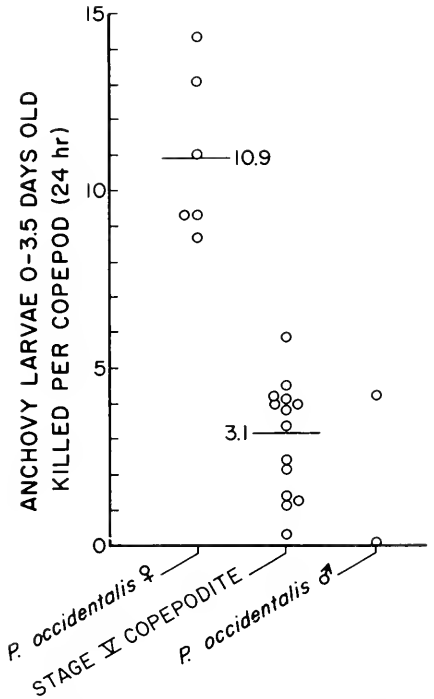

FIGURE 16.—Predation by *Pontellopsis occidentalis* on anchovy larvae 0 to 3.5 days old. Each horizontal line indicates the mean value of the experiments shown with open circles; 30 larvae were provided to 1 to 6 copepods in 3500 ml at the beginning of each experiment.

measure the rate of ascent of anchovy eggs to determine the maximum depth at which spawning could occur and yet insure the presence of yolk-sac larvae at the surface. In La Jolla seawater, salinity 33‰ and 17° C, anchovy eggs rise 5 cm/min or 3 m/hr. Thus, with time from spawning to hatching at 2 days, eggs spawned as deep as 144 m would hatch at or near the surface of the ocean, although spawning that deeply is rare (Ahlstrom, 1959). During development and, as they use up their yolk, anchovy larvae become almost neutrally buoyant and start to sink very slowly in laboratory containers. Even so, after 2 days of development, 50% of the laboratory-reared larvae were still above the 30 to 40 cm depth.

Ahlstrom (1959) reported closing-net captures of anchovy larvae at a variety of stations and depths in the California Current off California and Baja California. He has kindly provided us with length distributions of anchovy larvae taken at two stations, a night station, 5206-90.28, where over 500 larvae were taken, and a day station, 5504-120.50, where over 5000 larvae were captured. The length of the larva is roughly indicative of its age (Kramer and Zweifel, 1971), and we have tabulated the depth distribution of anchovy larvae at these stations by length and age (Table 2).

The depth distributions by age and length of anchovy larvae indicate that 50% or more of anchovy larvae up to 3 weeks old are above 10 m in depth. Fifty percent of the youngest class, 3 to 4.5 mm and 1 week old or less, were above 3.5 m during the day, and were slightly more than 2 m deep in the night. Ahlstrom's data also suggest that larvae of the Pacific sardine, *Sardinops caerulea*, Pacific mackerel, *Scomber japonicus*, and jack mackerel, *Trachurus symmetricus*, all pelagic fish of the California Current, may be similarly distributed.

Labidocerans are not diurnal vertical migrants and seem to be confined near the surface of the sea. Oblique tows with a plankton recorder (Longhurst et al., 1966) were taken in an area where *Labidocera trispinosa* and anchovy larvae are known to occur. The results are shown in our Table 3. The volume of each discrete sample at a particular depth was small (4-6 m^3) and the zeros may simply indicate relatively low abundance below the surface. Both tows, taken a day apart in close proximity to one another, showed that *L. trispinosa* was mainly present above 10 m, as were anchovy larvae. Simultaneously 10-min neuston tows were taken which filtered 463 m^3 no deeper than 30 cm at the surface of the sea (Table 4). The large number of *Labidocera trispinosa* and anchovy larvae in these tows suggests that the upper 30 cm of the ocean

TABLE 2.—Depth distribution of anchovy larvae by age and length at two stations, 5504-120.50 (day) and 5206-90.28 (night); collected by Ahlstrom (1959).

Average depth of catch (m)	Length and age of larvae							
	3.0-4.5 mm 0-7 days old		5.0-7.5 mm 8-14 days old		8.0-11.0 mm 15-21 days old		11.5+ mm 21+ days old	
	no.	% of total	no.	% of total	no.	% of total	no.	% of total
Station 5504-120.50 (day)								
2	515	42	505	17	283	37	64	36
7	317	26	820	28	216	28	52	29
18	138	11	596	20	78	10	31	17
27	96	8	138	5	31	4	5	3
44	105	9	509	17	62	8	19	11
60	61	5	324	11	95	12	7	4
74	1	0	70	2	1	0	0	0
Total	1233		2962		766		178	
Station 5206-90.28 (night)								
2	65	50	52	40	44	20	35	36
7	54	42	48	37	73	34	25	26
17	10	8	15	12	50	23	14	14
27	0	0	15	12	49	23	23	24
Total	129		130		216		97	

TABLE 3.—Vertical distribution of *Labidocera trispinosa* adults and anchovy larvae at 32°55.1′ (Station 1 at 0100) June 3, 1970, and 32°45.8′ (Station 2 at 2340) June 3. Volume of water filtered for each discrete depth sample in oblique tows were: Station 1, 6 m^3; Station 2, 4.35 m^3. No *L. jollae* or *Pontellopsis occidentalis* individuals were caught in these samples.

Depth (m)	Number of *L. trispinosa* adults	Number of *Engraulis mordax* larvae
Station 1		
0- 3	13	5 (7-11 mm long)
3- 7	0	1
7-15	0	1
15-60	0	0
Station 2		
0- 2	4	17 (7-10 mm long)
2- 7	17	11 (9-10 mm long)
7-15	2	1
15-25	0	1
25-60	0	0

TABLE 4.—Numbers of *Labidocera jollae*, *L. trispinosa*, *Pontellopsis occidentalis*, and *Engraulis mordax* eggs and larvae taken in 463 m^3 within 30 cm of the surface simultaneously with the oblique tows described in Table 3.

Species	Station 1	Station 2
L. trispinosa	1152	6400
L. jollae	336	104
P. occidentalis	112	128
E. mordax	226 (5-10 mm long)	36 (10-12 mm long)

is the area which should be examined for further elucidation of this predator-prey relationship.

The observations presented in this paper indicate that marine copepods may be effective predators on larval fish, at least in the sense that a predator need not devour its prey but is equally effective if it injures it mortally. Young pelagic fish larvae are particularly susceptible to biting zooplankters because they have an extremely thin skin and are unable to survive once the skin is punctured. Pontellid copepods, in particular *Labidocera* spp., appear to have a well-developed vibration sense which serves to orient the copepod toward its swimming prey although this is preceded by random searching. A fish larva with its beating tail provides the right stimulus to the copepod to initiate an attack when the latter is close enough to detect the beat.

We have called attention to the vertical distribution of *Labidocera* in the sea and the apparent co-occurrence of larval anchovies in the same depth stratum. Unfortunately, quantitative data on the density of predatory copepods or other zooplankters as related to fish larvae have yet to be made. It is our opinion that pontellid copepods and fish larvae are concentrated in the upper few meters and probably the upper few centimeters of the sea and that observations of this oceanic fine structure may reveal densities of fish larvae to copepods which would implicate predatory copepods (and possibly other zooplankters) as important causes of larval fish mortality.

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TROPHIC INTERACTION BETWEEN THE SEA STAR *Pisaster giganteus* AND THE GASTROPOD *Kelletia kelletii*

RICHARD J. ROSENTHAL[1]

ABSTRACT

The sea star *Pisaster giganteus* and the gastropod *Kelletia kelletii* are conspicuous inhabitants of the sublittoral zone off San Diego, Calif. Diving observations over a period of 2½ years indicate that the two species are trophically interrelated. *P. giganteus*, an opportunistic predator, and *K. kelletii*, a carnivorous scavenger, have been observed feeding together on common food items. The sea star appears to be a major predator of the whelk, even though *K. kelletii* made up less than 10% of the diet of the sea star. The whelk does not display an avoidance response in the presence of *P. giganteus*. Coexistence between the two species is believed possible as long as *K. kelletii* does not become more preferred prey of the asteroid.

Available information on the behavioral responses of marine mollusks in the presence of predatory sea stars has increased markedly within the past few decades (Bullock, 1953; Feder, 1963, 1967; Margolin, 1964a, 1964b; Feder and Christensen, 1966; Montgomery, 1967). However, most of these investigations have been limited to laboratory or intertidal observations. Except for a recent study by Mauzey, Birkeland, and Dayton (1968), the interactions between mollusks and sea stars in the eastern Pacific subtidal waters have not been investigated. Direct sublittoral behavioral observations off the west coast of north America have been hampered by cold water and limited underwater observation time.

Assessment of predator-prey relationships between subtidal organisms has been limited mainly to recording interactions between two organisms under laboratory conditions. A species-specific avoidance reaction or escape response by a mollusk to a sea star is considered one indication of a predator-prey relationship. The evolution of such responses, and the recognition of chemical stimuli emanating from either organism, suggests a long standing predator-prey association. Mauzey et al. (1968) found, however, that biochemical similarities between a predator and other organisms could cause a prey species to avoid the nonpredatory species as well as the predator.

Gastropods which displayed no avoidance responses in the presence of specific sea stars have been observed by Bullock (1953), Margolin (1964b), and Feder (1967). Bullock (1953) even suggested that nonresponsive mollusks are characteristic of ecological situations where starfish predation on these species must be rare.

This paper examines laboratory and field data obtained on the behavioral interactions between the sea star *Pisaster giganteus* (Stimpson) and the gastropod *Kelletia kelletii* (Forbes). Included are observations on the feeding, species-specific responses, and predator-prey interaction between the two species.

P. giganteus is reported from Vancouver Island, British Columbia, to northern Baja California, Mexico (Fisher, 1930), while *K. kelletii* has been found from Santa Barbara, Calif., to San Quintin Bay, Baja California, Mexico (Abbott, 1954). Both species are conspicuous and abundant inhabitants of the nearshore subtidal reefs off southern California. Bathymetric distribution appeared to be somewhat similar for *K. kelletii* and *P. giganteus* off San Diego County,

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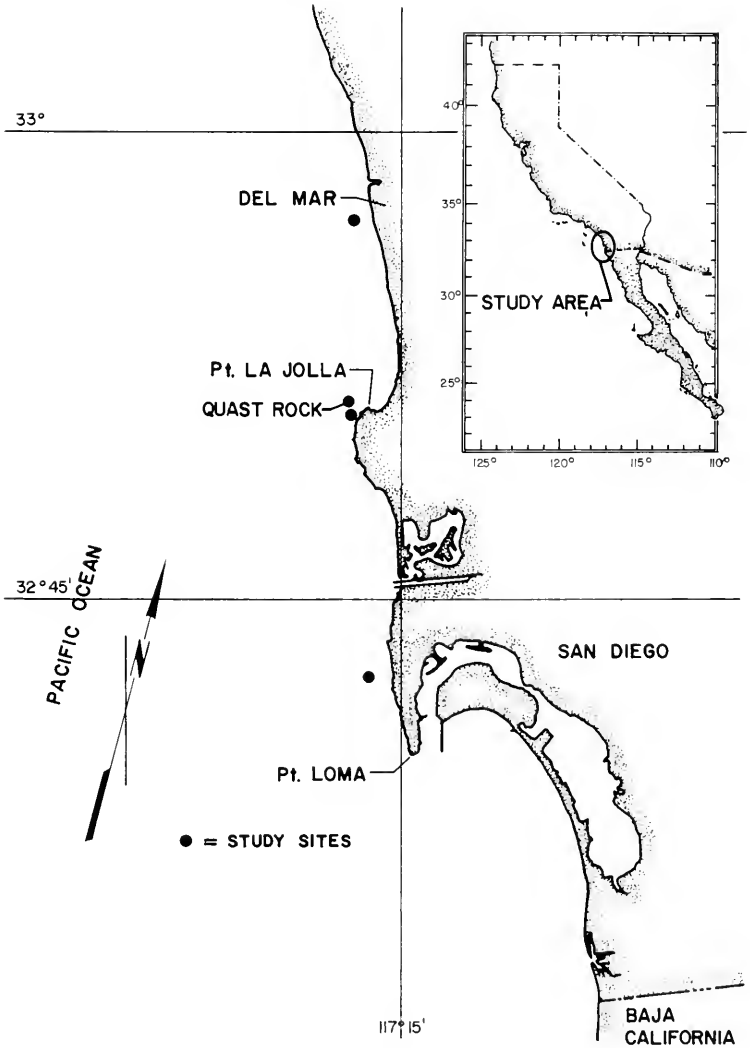

FIGURE 1.—Location of the four subtidal observation areas off San Diego County, Calif.

with the greatest concentrations of each between 2 and 40 m in depth.

The laboratory portion of the study was conducted in the experimental seawater aquarium of the National Marine Fisheries Service, Fishery-Oceanography Center, La Jolla, Calif. I made direct subtidal observations during daylight hours while scuba diving off San Diego County during the period January 1968-July 1970.

DESCRIPTION OF SUBTIDAL STUDY AREAS

Four widely separated nearshore locations within San Diego County were selected as field study sites (Figure 1). These sites were selected because they varied in depth, substratum, and species composition.

DEL MAR

The study area was located within a stand of giant kelp, *Macrocystis pyrifera*, which lies in 15 to 20 m of water about 1 km offshore from Del Mar, Calif. (lat 32°57′ N, long 117°16′ W). The kelp bed is characterized by having a relatively flat sea bottom with intermittent sand patches and low profile siltstone ledges. The sea floor is relatively homogeneous in appearance except for the occurrence of these ledges, which are less than 1.5 m in height. *Pterygophora californica*, a low standing brown algae, occurs abundantly on the seaward edge of the *M. pyrifera* bed.

POINT LA JOLLA

The observation site off Point La Jolla (lat 32°51′ N, long 117°16′30″ W) was between 150 and 300 m due west of Point La Jolla. The area is characterized by large boulders and undercut sandstone ledges. It is a topographically heterogenous substrate containing many microhabitats. Portions of the area contain large sandstone formations which rise vertically to within a few meters of the sea surface. The tops of these formations are often covered by surf grass, *Phyllospadix torreyi*. The observation area ranged between 5 and 16 m deep because of such pronounced vertical changes in relief. Scattered throughout the area are two species of perennial brown algae, *Egregia laevigata* and *Eisenia arborea*.

QUAST ROCK

Quast Rock is situated on an offshore reef about 630 m northeast of Point La Jolla (lat 32°51′30″ N; long 117°17′ W). The observation area encompassed approximately 225 m^2 of this reef. The rock is a sandstone formation with a deep undercut and a cave on the northern end. The substratum supports an extremely diverse benthic invertebrate fauna, largely because of the complexity of the habitat. The area is devoid of giant kelp and only two species of brown algae, *Cystoseira osmundacea* and *Agarum fimbriatum*, were common along the reef. The observation area ranged from 17 m on the top of the rock to approximately 27 m on a lower terrace.

POINT LOMA

The study site was located approximately 1.5 km offshore from Point Loma, Calif. (lat 32°42′ N; long 117°16′ W). The area is within a *M. pyrifera* stand and the bottom is between 13 and 18 m deep. The substratum is predominantly rock, siltstone, and sand. Portions of the bottom are interrupted by channels and low relief ledges. The shade area under the giant kelp canopy supports an algal undergrowth composed primarily of *P. californica*, *C. osmundacea*, and *Laminaria farlowii*.

FEEDING BEHAVIOR OF *Pisaster giganteus*

P. giganteus appears to be an opportunistic predator on the nearshore subtidal reefs off San Diego County. It feeds primarily upon live animals, although it has been observed scavenging on dead fishes and invertebrates. Thirty-two identifiable species of invertebrates, with pelecypods and gastropods making up about 80%

of the total, were seen being preyed on by this sea star at the four study areas (Table 1). The diet of *P. giganteus* is remarkably variable or generalized over the four subtidal observation areas, but within each habitat the diet is more

TABLE 1.—A list of prey species eaten by *Pisaster giganteus* and *Kelletia kelletii* from January 1968 to July 1970.

Prey (Class)	*Pisaster giganteus*	*Kelletia kelletii*
Anomia peruviana (pelecypod)	+	
Astraea gibberosa (gastropod)	+	
Astraea undosa (gastropod)	+	+ (S)[1]
Balanus tintinnabulum (crustacean)	+	+
Botula (Adula) falcata (pelecypod)	+	+
Bursa californica (gastropod)		+ (S)
Ceratostoma nuttallii (gastropod)	+	
Chama pellucida (pelecypod)	+	+ (S)
Conus californicus (gastropod)	+	+
Diopatra ornata (polychaete)	+	+
Hinnites multirugosus (pelecypod)	+	+ (S)
Jaton festivus (gastropod)	+	+ (S)
Kelletia kelletii (gastropod)	+	
Lithophaga plumula (pelecypod)	+	+
Loligo opalescens (cephalopod)	+ (S)	+ (S)
Maxwellia gemma (gastropod)	+	
Mitra idae (gastropod)	+	
Mytilus californianus (pelecypod)	+	
Mytilus edulis (pelecypod)	+	
Netastoma rostrata (pelecypod)	+	
Ostrea lurida (pelecypod)	+	
Panulirus interruptus (crustacean)	+ (S)	+ (S)
Paralabrax nebulifer (osteichthyes)	+ (S)	+ (S)
Parapholas californica (pelecypod)	+	+ (S)
Pelagia panopyra (scyphozoan)	+ (S)	
Phyllochaetopterus prolifica (polychaete)		+
Pisaster giganteus (asteroid)		+ (S)
Platyodon cancellatus (pelecypod)	+	+ (S)
Pododesmus cepio (pelecypod)	+	+
Pterynotus trialatus (gastropod)	+	
Pyura haustor (ascidian)	+	
Serpulorbus squamigerus (gastropod)	+	
Sphyraena argentea (osteichthyes)	+ (S)	+ (S)
Strongylocentrotus franciscanus (echinoid)	+ (S)	+ (S)
Strongylocentrotus purpuratus (echinoid)	+ (S)	+ (S)
Styela montereyensis (ascidian)	+	+
Taliepus nuttallii (crustacean)		+ (S)
Ventricolaria fordii (pelecypod)	+	+ (S)

[1] (S) = Scovenge.

selective or restricted (Figure 2). Variations in the diet of *P. giganteus* with each habitat or microhabitat are attributed to the availability and abundance of prey organisms, and preferences in the feeding behavior of the sea stars in these four locations.

Predator success may, in many instances, be dependent upon the predator's ability to feed upon what is available in a given habitat. The prey must be abundant enough to be utilized as a food source and the predator must be capable of selecting these forms. Variability in diet

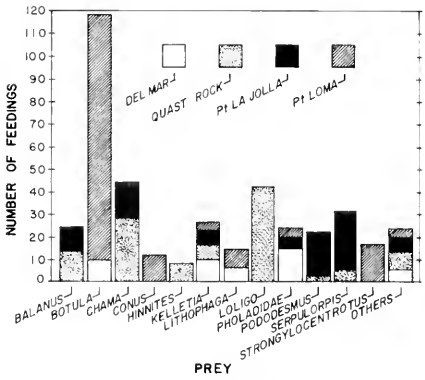

FIGURE 2.—Comparison of the feeding behavior of *Pisaster giganteus* as observed in the four study sites from August 1969 to July 1970.

with changes in sea star habitat has been discussed by Mauzey et al. (1968). Feder (1959) suggested that differences in the diet of the sea star *P. ochraceus* can largely be attributed to changes in prey availability.

Evidence for opportunistic or adaptable predation by *P. giganteus* is drawn from variability in diet within each habitat and a temporary alteration in the feeding behavior of the Quast Rock sea star population. On February 20, 1970, 12 out of 43 *P. giganteus* observed on or around Quast Rock were found to be feeding. Six of these individuals were eating *Chama pellucida;* two, *Balanus tintinnabulum;* two, *Serpulorbis squamigerus;* and one, *Pyura haustor.* Alteration in feeding was observed with the sudden appearance of a potential food source during the spring of 1970. Thousands of spawning squid, *Loligo opalescens,* were observed in the proximity of Quast Rock on March 12, 1970. Spawning had been reported to occur each year in the La Jolla area (Fields, 1965), although "spent" individuals had not been sighted in the Quast Rock location during the previous 12 months. Numbers of dying or dead squid were lying along the bottom off Quast Rock. A total of 52 *P. giganteus* was examined around Quast Rock; of these,

42 were feeding on dead *L. opaleseens*. The *P. giganteus* population had temporarily switched from active predators to scavengers with the availability of this abundant, though temporary, food source. I returned to the study site on April 10, 1970, and noted 6 out of 41 *P. giganteus* to be feeding equally on *C. pellucida*, *B. tintinnabulum*, and *Hinnites multirugosus*. With the dead squid no longer present, the sea star population returned to feeding on the available prey organisms of the reef.

Feeding preference experiments by Mauzey et al. (1968) and Landenberger (1968) indicated that the sea star *P. ochraceus* preferred mussels to alternative food items offered the sea stars. Landenberger (1968) also showed that *P. giganteus* preferred the mussels *Mytilus edulis* and *M. californianus* to five other molluskan species. Additional observations by Paine (1969) suggest that few other prey are consumed as long as mussels are readily available to *P. ochraceus*. However, mussels were rarely available to *P. giganteus* in the four subtidal locations examined. If *P. giganteus* has a specific food preference in these subtidal locations, then it must be adaptable or capable of change, since each habitat or microhabitat varies somewhat in prey availability.

P. giganteus appears to exhibit a feeding preference for prey that is either immobilized or sedentary in habit, since these organisms were eaten more frequently than motile forms. Attached or boring bivalves, balanoid barnacles, and sessile tube-dwelling mollusks were "preferred" or eaten most often by *P. giganteus*.

P. giganteus preys on *K. kelletii* more often than any other motile gastropod, and yet the whelks do not appear to be eaten in proportion to their abundance or accessibility in these locations. Abundance and distribution of *K. kelletii* was determined by random sampling along 150 m transect lines in two of the locations. The following densities were determined: Del Mar 1.82/m^2, and Point Loma 0.73/m^2. Distribution and movement of *K. kelletii* is, at least, sometimes nonrandom, for the whelks were found to be in an aggregated distribution pattern in each of these areas during late spring of 1970. The distribution pattern of a motile organism may reflect spawning or feeding interactions; therefore, these results were compared with and found to coincide with earlier data obtained from the Del Mar kelp bed during the fall of 1968. During the reproductive season (Rosenthal, 1970), numbers of *K. kelletii* occur in communal spawning groups, and yet predation by *P. giganteus* did not appear to increase with the availability of the whelks in these locations.

FEEDING BEHAVIOR OF *Kelletia kelletii*

K. kelletii is basically a carnivorous scavenger, although it has been observed feeding on live sedentary polychaetes. As a scavenger, it appears to be attracted to almost any injured or dead animal occurring on the sea floor (Table 1). Often, large numbers of *K. kelletii* have been observed moving towards and/or feeding upon a common food item in subtidal areas. The food-finding ability of *K. kelletii* by distance chemoreception has, on more than one occasion, been a nuisance to spiny lobster fishermen in some areas off southern California. These fishermen usually bait traps with dead fish to attract the spiny lobster *Panulirus interruptus*. Many times, however, a single lobster trap may contain dozens of *K. kelletii* which were attracted to the trap by the "scent" of the bait.

K. kelletii feeds with an extensible muscular proboscis which can be extended from the head region during feeding. Food is ingested by a muscular sucking action of the proboscis and a rasping of the radula. The proboscis is capable of extending approximately twice the length of the whelk's shell; it is this extension which allows *K. kelletii* to reach food items in depressions or within the substratum. Pearce and Thorson (1967) found that the proboscis of the gastropod *Neptunea antiqua* can be everted and may be extended to 2¼ times the length of the snail's own shell.

The proportion of the whelk's diet which results from preying on live animals versus scavenging on carrion or dying organisms was not determined. An aggregation of *K. kellctii* feeding on a dead fish or mollusk attracts the attention of an underwater observer more often than

FIGURE 3.—*Pisaster giganteus* and *Kelletia kelletii* jointly feeding on a date mussel, *Botula* (*Adula*) *falcata*. Note: The sea star's tube feet are touching the whelk's foot.

an isolate feeding. In many instances the snails were believed to be feeding since the proboscis was extended into a hole or within the substratum, and yet rarely was the food item identified.

Most of the scavenger feedings by *K. kelletii* attract more than one individual. In one instance, 85 *K. kelletii* were clustered around and feeding on a dead bass, *Paralabrax* sp., off Point Loma. Aggregate feedings on carrion or dying animals appeared to be the usual mode of feeding in these subtidal areas.

CONVERGENT FEEDING BEHAVIOR

A unique and yet reoccurring behavior pattern has been observed in those subtidal habitats where both species are found. *P. giganteus* and *K. kelletii* converge and attempt to feed on a common prey organism at the same time (Figure 3). Usually *P. giganteus* began to feed first, with the whelks grouped around the sea star (Figure 4). In many instances *K. kelletii* were

FIGURE 4.—Convergent feeding behavior between *Pisaster giganteus* and *Kelletia kelletii* 21 m underwater off Point La Jolla, Calif. The sea star is eating a *Chama pellucida*, while three whelks are attempting to get to the prey.

partially underneath or even crawling over the sea star attempting to get to the food item, and at times a feeding sea star would also be grasping from one to four *K. kelletii*. A few of these interspecific feedings were quite distinct in appearance, especially when both species were scavenging larger prey such as dead fishes. Three *P. giganteus* and 28 *K. kelletii* were observed feeding simultaneously on a dead barracuda, *Sphyraena argentea*, off Quast Rock on December 31, 1968. However, most of the feeding convergences observed were not as obvious as this; usually they involved only a single sea star and two or three whelks. Convergent feeding behavior has been noted 65 times in these four locations over a 2-year period. On one occasion, five separate convergent groups were observed within a 400 m^2 area off Point Loma.

Direct competition for food was most evident when both species were scavenging carrion or moribund organisms. However, in situations where *P. giganteus* had captured or killed live prey, *K. kelletii* was rarely observed with its proboscis extended into the prey. It is probable that feeding by the whelk usually takes place on a secondary basis after the sea star has departed. The percentage of prey that is left uneaten by *P. giganteus* following a feeding is unknown.

These convergent feeding groups were not limited to *K. kelletii* and *P. giganteus*. *K. kelletii* has been observed feeding interspecifically with two other sea stars, *Dermasterias imbricata* and *Pisaster brevispinus*. Other scavenging or carnivorous types of epibenthic invertebrates which have been found in these sea star-whelk feeding groups include the gastropod *Mitra idae* and the hermit crab *Paguristes ulreyi*.

PREDATION ON *Kelletia kelletii*

There have been few natural predators of *K. kelletii* reported in the literature. The moon snail, *Polinices lewisii*, was observed by MacGinitie and MacGinitie (1949) to have drilled and eaten a live *K. kelletii* in a laboratory tank. Juvenile *K. kelletii* were found in the stomachs of young pile perch, *Rhacochilus vacca*, by Limbaugh (1955). Three predators of *K. kelletii* observed during diurnal hours in these subtidal locations were *Pisaster brevispinus*, *P. giganteus*, and the cephalopod *Octopus bimaculatus*. However, only predation by *P. giganteus* is considered at this time.

P. giganteus is a major predator of *K. kelletii* on the nearshore reefs that I studied. During the 2½-year study period, 42 separate feedings were observed in which the sea star was in the act of digesting a whelk; and 53 other times, *P. giganteus* were found attacking *K. kelletii*. Five separate attacks by *P. giganteus* on *K. kelletii* were observed in a single dive off middle Coronado Island, Baja California (lat 32°25′ N, long 117°16′ W) on December 3, 1969.

The sea star usually fed by attaching tube feet to the substratum and the shell and the operculum of the *K. kelletii*. *P. giganteus* was usually observed in a humped or arched position while attacking and feeding on the whelk. In this feeding position, a sea star can bring into play a greater number of tube feet and exert maximum pull on the operculum and shell of the whelk while still remaining attached to the substratum (Feder and Christensen, 1966). *K. kelletii* were not swallowed or ingested whole by *P. giganteus;* instead, digestion appeared to take place extraorally as is the case with other food items. The whelk's operculum was either torn away or pulled out of the shell opening by the tube feet, and the sea star's stomach was inserted into the shell. Bullock (1953) suggested that predation on the intertidal snail *Acanthina spirata* by carnivorous asteroids was slight, and that possibly possession of a heavy operculum accounted for the nonresponsive behavior the snail displayed in the presence of predatory sea stars. The operculum of *K. kelletii* does not eliminate sea star predation; however, the structure apparently does increase the time necessary for an asteroid to complete the feeding process.

The *K. kelletii* which were attacked by *P. giganteus* ranged between 18 and 120 mm in shell length (siphonal canal to the apex); however, approximately 70% of these feedings involved *K. kelletii* greater than 60 mm in length. This size class, greater than 60 mm, was com-

posed primarily of sexually mature individuals (Rosenthal, 1970). *K. kelletii* less than 40 mm in length were rarely preyed on by *P. giganteus*. These smaller individuals are more secretive in habit than larger mature *K. kelletii*, and they characteristically burrow in the substratum. Possibly the burrowing and cryptic behavior of smaller *K. kelletii* decreases sea star predation by reducing the number of contacts with predatory *P. giganteus*. *K. kelletii* is considered to be what Paine (1969) referred to as "secondarily preferred prey," since invertebrate species other than *K. kelletii* were found to be numerically more predominant in the diet of *P. giganteus* at these four locations (Figure 2).

Seasonal changes in *K. kelletii* predation by *P. giganteus* was not considered to be a factor in these subtidal regions. *P. giganteus* was observed feeding on whelks throughout the year with no noticeable change in the incidence of predation.

RESPONSE OF *Kelletia kelletii* TO *Pisaster giganteus*

From the number of reports of mollusks responding to predatory sea stars (Feder and Christensen, 1966), I initially expected to observe an avoidance reaction by *K. kelletii* in the presence of *P. giganteus*. To test this, 150 *K. kelletii* between 15 and 138 mm in shell length were brought into the laboratory and maintained in either standing or circulating seawater. The seawater temperature ranged between 16.0° and 19.9° C throughout the entire experiment. *K. kelletii* were tested for any reaction which might be exhibited in the presence of, or while touching, *P. giganteus*. At times, the whelks responded by siphon extension, shell rocking, or twisting, and a slow sliding movement away from or in the direction of *P. giganteus*. Many times no shell movement was noted within a 10-min period. Tests were conducted on whelks that were inactive and on others that were moving, feeding, or spawning. Each *K. kelletii* was used only once or twice so that continual contact with the sea star would not affect the whelk's reaction. At no time did I note an escape or avoidance response by *K. kelletii* in the presence of the sea star.

Field observations were similar to those in the laboratory; either *K. kelletii* did not respond or, at times, they actually were attracted to *P. giganteus*. The two species were usually found close to one another on these subtidal reefs, and contacts between the two probably are frequent. During convergent feeding, the two species touched or even crawled over one another; however, at other times the *K. kelletii* and *P. giganteus* occurred within a few centimeters of each other even though neither species was engaged in feeding. Paine (1969) found a perplexing intimacy of association between three intertidal gastropods and their major predator, the sea star *P. ochraeeus*. In contrast to these two situations, Bullock (1953: 137), stated that "In those seashore situations where predatory starfish and gastropods both occur, it is notable that the two are generally not seen close together."

One of the routine field experiments was to pick up a *P. giganteus* underwater and place it on or within a few centimeters of an individual or group of *K. kelletii*. On only one occasion was a reaction exhibited in *K. kelletii* out of the hundreds of attempts to stimulate an active response. In this one instance, a group of 11 *K. kelletii*, which appeared to be searching for food, was encountered 20 m underwater off Del Mar on November 6, 1968. A large *P. giganteus* was placed approximately 30 cm from the group of whelks, and within a few minutes the sea star approached the *K. kelletii*. All of the whelks moved away from the approaching sea star; however, two of the *K. kelletii* were captured by the sea star while the others moved off in a similar direction. This reaction appeared to be an avoidance response although it could have been only random or chance movement on the part of the whelks, regardless of the presence of the sea star.

CONCLUSION

The existence of a predator-prey relationship between a gastropod and a sea star is not unusual; however, the continual nonresponsive or

at times even attractant behavior the whelk displays in the presence of this potential predator appears to be unique. *P. giganteus* and *K. kelletii* are trophically interrelated. Trophic interaction is based on a predator-prey relationship and similarities in the diet of both species. The prey species consumed by *P. giganteus* and *K. kelletii* are similar; however, the method of feeding, as well as the physical condition of the prey, is usually dissimilar.

These observations leave many unanswered questions on the behavioral relationship between a potential prey organism and a predatory sea star. *K. kelletii* is preyed upon by *P. giganteus;* however, the whelk does not respond to sea star contact as has been reported in other gastropod-asteroid interactions. The sea star is a major predator of *K. kelletii*, although the whelk makes up less than 10% of the sea star's diet. The factors which limit predation of *K. kelletii* by *P. giganteus* are unknown, since both species are such conspicuous and extremely abundant inhabitants of the sublittoral zone off San Diego County. Feder (1963) studied intertidal sea star predation on gastropods and noted that species which exhibited avoidance responses in the presence of asteroids were not preyed upon in proportion to their abundance or availability in the intertidal. *K. kelletii* does not exhibit avoidance or escape responses in the presence of *P. giganteus*, and yet it does not appear to be eaten in proportion to its accessibility or abundance in subtidal areas off San Diego. A general feeding preference by the sea star for attached or nonmotile prey is thought to limit predation on *K. kelletii* in these locations. The cryptic and burrowing habits of juvenile *K. kelletii* may further reduce predation on the smaller whelks by limiting the number of contacts with predatory sea stars. Paine (1969) suggested that coexistence between a major predator and prey is possible as long as the prey species does not reach a more preferred status in the diet of the predator.

It can only be speculated that the escape response of *K. kelletii* in the presence of the sea star has either not evolved or possibly was lost through continual contact and convergence on a similar trophic level. Perhaps other behavioral activities such as feeding may be of greater selective value to *K. kelletii* than is a species-specific avoidance response. Quite possibly the whelk benefits more by not actively avoiding *P. giganteus* than it would by continually running from this potential predator. The chances for *K. kelletii* to feed on moribund or dead organisms would be increased, since both species are attracted to these food items.

SUMMARY

1. Trophic interaction between the sea star *P. giganteus* and the gastropod *K. kelletii* is based on a predator-prey relationship and similarities in the diet of both species.

2. *P. giganteus*, a highly opportunistic predator, was observed feeding on 32 identifiable species of invertebrates. The sea star exhibited a preference for prey which was either immobilized or sedentary in habit, since these forms were preyed upon most heavily.

3. *K. kelletii*, a carnivorous scavenger, usually feeds on moribund or dead organisms that it finds resting on the sea floor.

4. *K. kelletii* and *P. giganteus* feed together on common food items. Convergent feeding between these two species was a recurring behavioral pattern which was observed repeatedly in the four subtidal areas.

5. *P. giganteus* is the major identifiable predator of *K. kelletii* off San Diego County; however, the whelk makes up less than 10% of the prey observed to be captured by the sea star. The whelk is believed to be of secondary importance as food of the sea star, since invertebrates other than *K. kelletii* were utilized more often.

6. *K. kelletii* did not display a species-specific avoidance or escape response in the presence of, or while in contact with, *P. giganteus*.

7. Both species appear to be highly successful and abundant organisms of the sublittoral zone, and coexistence is believed possible as long as predation by the sea star on *K. kelletii* is not excessive.

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VARIABILITY OF NEAR-SURFACE ZOOPLANKTON OFF SOUTHERN CALIFORNIA, AS SHOWN BY TOWED-PUMP SAMPLING

CHARLES P. O'CONNELL[1]

ABSTRACT

Variations in the density of near-surface populations of small copepods, large copepods, euphausiids, and chaetognaths are described for an area of 6,000 square miles off the coast of southern California from three cruises in the autumn of 1961 and two cruises in the autumn of 1962. Samples were collected with a towed pump at a depth of 5 m. Approximately 162 samples, each representing a 1-mile transect, were collected on each cruise.

Median densities for the cruises showed some significant differences for each species group. The frequency distribution of densities within the area on individual cruises varied from positive skewness at low general levels to relative symmetry at high general levels for the three crustacean groups, but was skewed at all levels for chaetognaths. Within sampling blocks of 20 square miles, the range of density varied with the median as $\log R = 0.35 + 0.8 \log M$. Range is greater than the median when the latter is less than 50, but less than the median when it is higher than 50.

Euphausiids and large copepods showed greater diurnal change than small copepods and chaetognaths.

Dry weight concentration of samples, averaged over all cruises, was 17.3 mg/m^3 for the day period (0600-1800) and 25.1 mg/m^3 for the night period. Most of the nighttime increase is attributable to the euphausiid group.

The three crustacean groups, and dry weight, showed significant inverse trends with temperature, but not with distance from land. The trends with temperature reflect events in 1961 but not in 1962.

These variations suggest that food potential of plankton for pelagic fishes may be appreciably greater than indicated by general averages for the area, depending on the degree of selectivity and orientation to small-scale features of distribution by the fishes.

Little is known about the effects of plankton variability on the distribution, movements, or rate of feeding of pelagic fishes which feed on plankton. It has been demonstrated experimentally (Ivlev, 1961) for some fishes that rate of feeding varies not only with average density but also with the degree of aggregation of food organisms in an area. Plankton density is known to vary diurnally (Cushing, 1951; King and Hida, 1954) as well as seasonally and annually, and there is evidence of aggregation in the variation for both small and broad spatial scales (Barnes and Marshall, 1951; Cassie, 1959, 1962, 1963). The plankton pump surveys reported here were undertaken to obtain information on variability and trends in variability for four plankton species groups commonly present in near-surface waters along the southern California coast. Though surveys were limited to the autumn seasons of 2 consecutive years, the data should be a useful guide in evaluating the food potential of near-surface plankton distributions in the region.

COLLECTION OF SAMPLES

Samples were collected with the towed pump and shipboard filtering system described by O'Connell and Leong (1963). The 1.9-cm ($^3/_4$-inch) orifice of the pump pointed forward to achieve a coring orientation, and the rate of pumping (98 liters/min) exceeded the passive coring rate to produce in effect a 5.8-cm (2-inch) diameter coring cross-section. Operation of the system was essentially a matter of leaving the pump in tow and running throughout a cruise pattern with the incoming water stream diverted to the scuppers except while traversing sampling blocks, at which time the flow was directed through the filtering apparatus. The stainless steel filtering screen (105μ mesh) retained

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virtually all organisms as small as 200μ in length (O'Connell and Leong, 1963) or 100μ in diameter (Leong, 1967). The upper size limit was less easily defined, but organisms as long as 14 mm were delivered at the filtering apparatus, though the large individuals were often mutilated.

Five cruises were carried out, three in September to November 1961 and two in the same period of 1962. For each cruise a pattern of 18 sampling blocks was selected from a possible 281 that covered an area of almost 6,000 square miles (Figure 1). To insure reasonably good coverage of the entire area, the population of blocks was divided into three approximately equal subareas and a set of six blocks was selected at random from each. The blocks were occupied by the shortest practical track from northwest to southeast. Each cruise pattern required about 2.5 days of vessel time.

Each sampling block was 51.8 km^2 (20 square miles) in area, the only exceptions being some of the blocks adjacent to the coast or to islands. Nine 1.6-km (1-mile) samples were collected at each block in a continuous series along two connecting sides (Figure 1, insert) and were preserved in Formalin for laboratory processing.

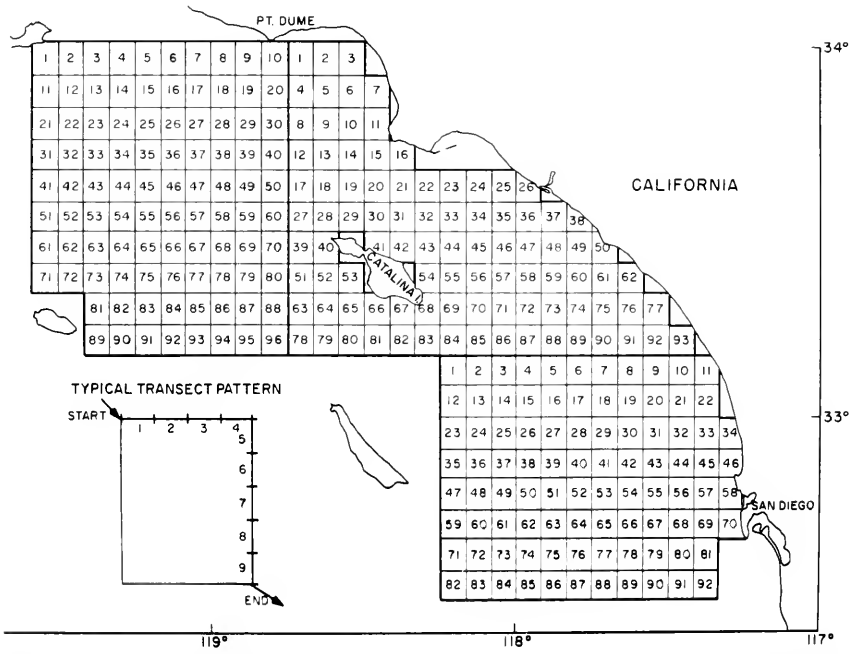

FIGURE 1.—The sampling area and entire population of sampling blocks. Six blocks were randomly selected from each of the three subareas for each cruise and occupied by the shortest route from north to south. Sampling blocks are 4 × 5 miles and the insert shows the manner of transect sampling. Four of the nine samples from each block were selected randomly for organism enumeration.

Consecutive 1.6-km samples were separated by shifting the incoming stream to a new filter every 6.5 min. Water volume, recorded for each sample from a meter in the incoming line, averaged 636.7 liters (standard deviation 47.8). Water temperature of the incoming stream was recorded at approximately the midpoint of each series of block samples. Continuous thermograph records indicated that surface water temperature did not change appreciably during the sampling of individual blocks.

SAMPLE PROCESSING

Four of the nine samples from each block were randomly selected for the estimation of numbers of organisms. Of the remaining five, one was chosen at random to be reserved for special purposes such as length and dry weight measurements of species groups, and the other four were pooled to obtain a dry weight value for the block. Estimates were standardized as quantities per m^3 on the basis of the actual volumes of water filtered in the samples.

Estimates for two size categories of copepods, 0.2 to 0.9 mm in length and all over 1.0 mm long, and for euphausiids and chaetognaths, were made by volumetric subsampling with replacement, i.e., each subsample was returned to the sample before the next subsample was drawn. The volumetric subsampling technique yields estimates of satisfactory precision only if organisms are randomly distributed in the sample container prior to the removal of each subsample. Simple stirring accomplished this for all species groups except the small copepods, probably because they were entangled in phytoplankton present in the samples. Random distribution was assumed to exist where the value s^2/x did not exceed $\chi^2/N\text{-}1$ for a series of subsamples (Holmes and Widrig, 1956). A random distribution was achieved for the small copepods by subjecting the sample to a few 1-sec bursts of rapid stirring in a Waring Blender.[2] However, because this treatment fragmented some larger organisms a two-step procedure was employed: all organisms except the small copepods were estimated by subsampling following gentle stirring; the sample was then agitated in the Waring Blender, after which the small copepods were estimated by subsampling.

Estimates for the small copepods were always based on subsample counts totaling 200 to 300 from the sample. With the assumption of random distribution, the number in the sample should in all cases be within 15% of the estimate for $p = 0.05$ (Holmes and Widrig, 1956). More than half of the sample estimates for the other three species groups were based on counts of 30 or more, for which the number in the sample should have been within 40% of the estimate. For the remainder, where numbers counted were low, examination was not extended beyond subsamples totaling one-third the volume of the sample container, 2,000 ml.

In addition to the four species groups counted, the samples contained larvaceans and small invertebrate eggs (0.15-0.35 mm diameter), sometimes in moderately high numbers. Larvacean tails and heads were separated, however, and invertebrate eggs were not readily distinguishable from the latter. Cladocerans and polychaetes were generally absent or low in number, though each occurred in high numbers in a few samples. Fish eggs occurred rarely and in low numbers.

SIZE OF ORGANISMS

Length measurements for 10 day samples (0600-1800) and 10 night samples are summarized in Table 1 and Figure 2. Measurements were total length except for euphausiids, which were measured from the carapace behind the eye to the junction of the abdomen and telson. Data from day and night samples were pooled for small copepods and chaetognaths but not for large copepods and euphausiids, which showed appreciable size frequency differences for the two periods.

The length-frequency distribution for the small copepod group, composed largely of naupliar and copepodite stages, is nearly symmetrical, with the mean and the median close to the midpoint of the predetermined size range (0.20-0.99 mm long). Almost one-third of the organisms

[2] The use of trade names is merely to facilitate descriptions; no endorsement is implied.

TABLE 1.—Length data for species groups.

	Mean length	Median length	Minimum length	Maximum length
	mm	*mm*	*mm*	*mm*
Small copepods	0.58	0.56	0.20	0.99
Large copepods:				
Day	1.94	1.90	1.00	5.30
Night	1.75	1.61	1.00	4.20
Euphausiids:				
Day	2.3	2.1	0.6	9.7
Night	5.7	5.9	1.1	10.6
Chaetognaths	6.4	5.5	2.0	14.5

were between 0.5 and 0.6 mm in length. It is possible that the decline in numbers below the median length was partly the result of increasing escapement with diminishing size, but the escaping fraction, known to be negligible for sizes above 0.4 mm, is assumed to be relatively small for sizes down to 0.2 mm (O'Connell and Leong, 1963). On this assumption the length-frequency distribution is considered representative for this size range of copepods.

The large-copepod group shows a modal shift to smaller sizes at night, although the size range and degree of skewness are not markedly different for the two periods. Organisms less than 1.5 mm in length were largely copepodites, while

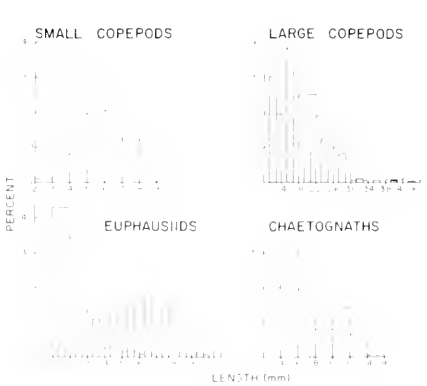

FIGURE 2.—Length-frequency histograms of organisms in four species groups, as determined from selected samples. For large copepods and euphausiids the wide bars show day frequencies and the narrow bars night frequencies.

those between 1.5 and 3.0 mm were adult *Calanus helgolandicus* and *Paracalanus* sp., with *Centropages* sp. also present in the night samples. Those larger than 3.0 mm were largely *Rhincalanus* sp. in both day and night samples.

The euphausiid group, which appeared to be composed largely of *Euphausia pacifica*, though *Nyctiphanes simplex* and *Nematoscelis difficilis* were also in evidence, showed a marked shift to larger sizes at night, obviously the result of vertical migration. It is apparent from the sizes involved that the day samples were composed mostly of larval stages and the night samples of larval stages and juveniles, with few if any adults. The largest individuals in the samples were considerably smaller than the maximum total length for the species, 25 mm (Boden, Johnson, and Brinton, 1955).

The pump samples did show some evidence of fragmentation of larger euphausiids, and for this reason the size frequency distribution for the night period might be slightly biased in favor of the smaller sizes, and estimates of numbers sampled might be a little low.

Fragmentation probably involved far more juveniles than adults. Samples from opening-closing nets 1 m in mouth diameter taken in spring and summer off southern California (Brinton, 1962) showed adults to be scarce or absent in the upper 10 m during the night as well as the day. Juveniles were predominant at this stratum. Even at depths where adults were most abundant at night—10 m in one case and 110 to 280 m in another—they were only one-fifth and one-tenth as numerous as juveniles. Nighttime oblique hauls with nets 1 m in mouth diameter off central Baja California indicated essentially the same kind of vertical distribution for euphausiid species in that area (Ahlstrom and Thrailkill, 1963). On the basis of such evidence it seems probable that the size-frequency distributions shown by the pump samples are reasonably representative of the day and night populations near the surface, though certainly not of the population in the entire water column.

The chaetognath group was composed largely, if not entirely, of *Sagitta euneritica*, and the size range is probably representative for the near-surface population sampled. The size range for

the species is 1.0 to 15.5 mm in length (Alverino, 1961), and the samples contained individuals from 2.0 to 14.5 mm long.

VARIATION BETWEEN CRUISES

Table 2 shows characteristics of the frequency distributions of all 1.6-km samples by cruise and period of the day for the four species groups. Median densities are lower than mean densities in every instance but one, the nighttime period for small copepods on cruise 5, indicating that distributions for virtually all arrays show some degree of positive skewness. Differences between cruises are described in terms of the medians to avoid undue effects of extreme values that can arise in moment measures on nonnormal populations.

Nighttime median densities are higher than daytime medians on all cruises for small copepods and euphausiids, and on all but the fifth cruise for large copepods. However, variation was such that day and night medians differed significantly ($p<0.05$, Tate and Clelland, 1957) in only one instance for small copepods, three instances for large copepods, and two instances for euphausiids. Though none of these groups can be considered to show consistently higher densities at night for the area as a whole, real differences occurred more often for the large copepods and euphausiids than for the small copepods. Day and night median densities for chaetognaths do not differ significantly for any of the cruises.

Daytime median densities do not differ significantly between cruises for chaetognaths or euphausiids, but those for small copepods and large copepods differ significantly ($p<0.05$) for about half the comparisons. Nighttime medians (Figure 3) show a pattern similar to the daytime sets for the two copepod groups but show, in addition, a number of significant differences between cruises for the euphausiids and one difference for chaetognaths. Median densities of the small copepods were significantly higher for

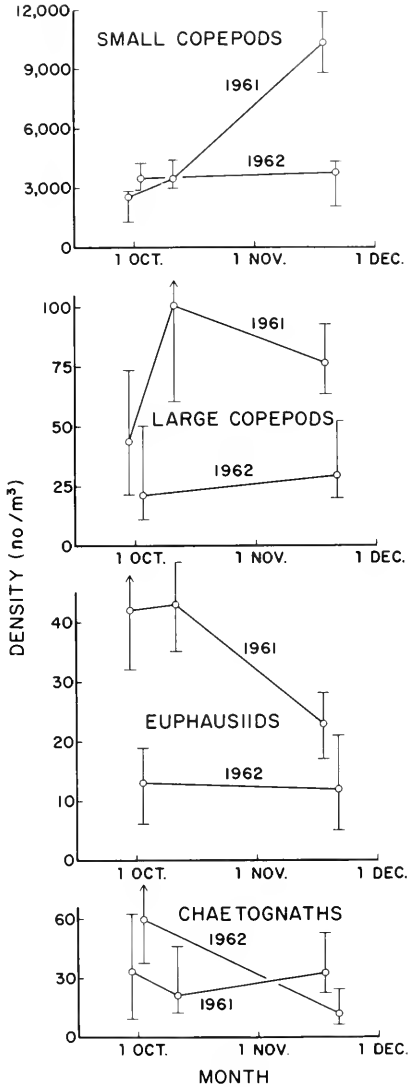

FIGURE 3.—The nighttime cruise medians for four species groups shown by cruise date. Vertical bars indicate the 95% confidence intervals.

TABLE 2.—Summary of density estimates (no/m³) of 1.6-km samples by cruise, constituent group, and period of day.

	Mean	s	Median	Confidence limits[1] $p = 0.95$ Lower	Upper	Minimum	Maximum
Cruise 1, September 26-28, 1961, N = 32 day, 40 night							
Small copepods							
Day	2,569.7	987.4	2,275.5	2,023	2,552	1,210	5,233
Night	2,983.9	1,356.2	2,542.0	2,252	2,792	1,075	7,103
Large copepods:							
Day	6.4	16.0	1.0	0	5	0	83
Night	72.8	95.5	42.5	22	73	0	500
Euphausiids:							
Day	6.7	9.4	3.0	2	8	0	38
Night	52.2	37.1	41.5	32	57	10	172
Chaetognaths:							
Day	84.2	122.9	21.0	8	105	2	488
Night	123.6	244.2	33.0	10	63	2	1,153
Cruise 2, October 9-11, 1961, N = 24 day, 40 night							
Small copepods:							
Day	3,379.0	1,773.1	3,034.5	2,217	3,527	1,550	7,532
Night	3,953.0	1,620.0	3,325.0	2,942	4,257	2,142	8,118
Large copepods:							
Day	47.3	104.5	1.0	0	17	0	440
Night	102.7	67.3	101.0	60	128	5	250
Euphausiids:							
Day	10.9	19.7	1.0	0	15	0	88
Night	48.2	31.7	42.5	35	50	7	145
Chaetognaths:							
Day	132.7	135.3	121.5	3	198	0	420
Night	51.8	61.9	21.0	13	47	2	280
Cruise 3, November 16-18, 1961, N = 28 day, 40 night							
Small copepods:							
Day	8,679.0	3,969.7	7,381.5	6,107	10,208	3,752	19,868
Night	10,577.8	3,060.7	10,281.0	8,673	11,812	4,948	17,568
Large copepods:							
Day	18.3	21.1	10.0	5	18	0	92
Night	83.0	42.9	77.0	63	95	10	183
Euphausiids:							
Day	15.5	13.9	13.0	5	22	0	48
Night	39.5	50.8	23.0	17	28	2	243
Chaetognaths:							
Day	73.5	104.5	36.0	32	42	12	470
Night	44.5	38.8	32.5	23	50	3	188
Cruise 4, October 1-3, 1962, N = 43 day, 26 night							
Small copepods							
Day	2,718.1	1,748.8	1,993.0	1,739	2,771	456	8,190
Night	3,524.1	1,087.1	3,504.0	2,854	4,179	1,477	5,560
Large copepods.							
Day	21.3	19.6	18.0	10	22	0	90
Night	33.6	29.5	21.5	11	50	3	99
Euphausiids:							
Day	8.2	9.1	4.0	3	10	0	39
Night	16.0	14.3	13.0	6	19	1	58
Chaetognaths.							
Day	116.7	164.6	57.0	35	133	9	993
Night	86.9	71.6	60.0	38	113	13	257
Cruise 5, November 19-21, 1962, N 32 day, 31 night							
Small copepods:							
Day	3,728.2	2,277.8	3,487.0	2,235	4,328	1,654	11,613
Night	3,505.3	1,962.1	3,750.0	2,054	4,223	796	9,476
Large copepods.							
Day	121.5	252.0	49.5	29	70	0	1,220
Night	41.1	35.6	30.0	19	52	1	154
Euphausiids:							
Day	9.8	11.0	6.5	1	13	0	48
Night	24.4	34.4	12.0	5	21	0	163
Chaetognaths:							
Day	32.2	20.8	28.5	19	41	3	94
Night	21.0	20.2	12.0	7	25	0	72

[1] Confidence limits are for medians (Tate and Clelland, 1957).

each successive cruise in 1961, and the median for the last cruise in this year was more than three times as high as any of the others. Median densities of the large copepods were two to four times as high for the last two cruises in 1961 as for the other cruises. Euphausiid medians were about twice as high for the first two cruises of 1961 than for the other cruises. The chaetognath median for the last cruise of 1962 was significantly lower than for the first cruise of that year, but no other differences can be distinguished.

In summary, the three crustacean groups showed real differences in density near the surface at night for the area as a whole. All showed differences of three to four times between the 2 successive years. The euphausiids and small copepods also showed real differences of about two and three times within the first of the 2 years.

VARIATION WITHIN THE AREA

The 1.6-km samples were taken in block clusters so that small-scale variability could be described in respect to variability over the entire area. The possibilities of comparison are limited, however, because parametric analyses were avoided. The necessary assumptions about frequency distribution of sample estimates could not be satisfied for the present data. Frequency distribution of block medians (or means) is variable between cruises.

FREQUENCY DISTRIBUTION OF BLOCK MEDIANS

Table 3 shows the frequency distribution of block medians for the day and night periods of each cruise. The distributions for each species group vary noticeably, but there are similarities

TABLE 3.—Frequency distribution of block medians by cruise and day (D) or night (N) period.

Class (no/m³)	1D	1N	2D	2N	3D	3N	4D	4N	5D	5N
Small copepods:										
1-3,000	6	7	3	4	--	--	8	3	4	4
3,001-6,000	2	3	2	5	--	--	3	4	4	5
6,001-9,000	--	--	1	1	5	4	--	--	--	--
9,001-12,000	--	--	--	--	1	2	--	--	1	--
12,000-15,000	--	--	--	--	1	4	--	--	--	--
Category	1	1	2	2	3	4	1	2	2	2
Large copepods:										
0	3	--	3	--	--	--	--	--	--	--
1-50	5	5	2	3	7	1	11	5	6	7
51-100	--	3	--	2	--	7	--	2	1	1
101-150	--	1	--	3	--	2	--	--	--	1
151-200	--	--	--	2	--	--	--	--	1	--
201+	--	1	1	--	--	--	--	--	1	--
Category	1	3	1	4	1	4	1	2	3	2
Euphausiids:										
0	2	--	2	--	1	--	2	--	1	--
1-25	5	2	3	2	5	5	9	6	8	7
26-50	1	3	1	4	1	3	--	1	--	1
51-75	--	3	--	3	--	1	--	--	--	--
76-100	--	2	--	1	--	--	--	--	--	1
101-125	--	--	--	--	--	--	--	--	--	--
126-150	--	--	--	--	--	--	--	--	--	--
151+	--	--	--	--	--	1	--	--	--	--
Category	1	3	1	3	1	2	1	2	1	2
Chaetognaths:										
1-50	5	6	2	7	5	7	4	2	8	9
51-100	1	2	1	1	1	2	3	2	1	--
101-150	--	--	--	1	1	1	3	2	--	--
151-200	--	--	2	1	--	--	--	--	--	--
201-250	1	1	--	--	--	--	--	1	--	--
251-300	1	--	1	--	--	--	--	--	--	--
300+	--	1	--	--	--	--	1	--	--	--
Category	1	1	3	2	2	2	3	3	2	2

among them suggestive of trends with the general level of density. To define the trends, the distributions for each species group were pooled into three or four categories on the basis of the extent of concentration in specific frequency classes and the extent of dispersion over all classes. Category designations are indicated in the table, and percentage frequency histograms are shown for each category in Figure 4. Number of blocks, number of night and day periods, and the range of cruise medians (from Table 2) are given for each histogram.

The histograms suggest the same kind of trend for the three crustacean groups: a shift from distribution almost entirely restricted to the lowest classes when general area level is low, through distribution of greater positive skewness for intermediate levels of area density, to symmetrical distribution as blocks of low median value disappear at the higher levels of area density. The chaetognath distributions were more difficult to classify, but it appears that frequency distribution is appreciably skewed at all levels of area density for this group.

The differences in the trends for the four species groups are also illustrated by the extent of the overlap between the distributions for the highest and lowest categories in the figures. There is no overlap for small copepods, perhaps 50 to 75% overlap for large copepods and euphausiids, and complete overlap for chaetognaths. These differences suggest that when the overall area median is at one extreme, the possibility of blocks with medians at the other extreme is greatest for the chaetognaths, least for the small copepods, and intermediate for the large copepods and euphausiids.

The existence of such trends in frequency distribution indicates that, at least for the crustaceans, no one statistical distribution would satisfactorily fit all the data sets; nor would any single normalizing transformation be uniformly effective for the different data sets. Without normalized distributions, even the interpretation of coefficients of variation would be difficult in comparing cruise periods. It may be noted, however, that when all frequency distribution categories are pooled, the distributions for the four species groups show similar degrees of skewness. The total block array for each species group is approximately normalized by log transformation.

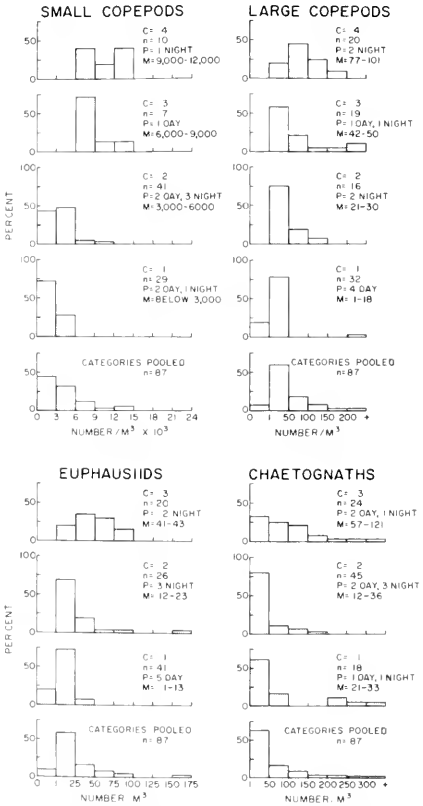

FIGURE 4.—Percent frequency distribution histograms for four species groups as pooled on the basis of similarity. Range of sample medians (M) (from Table 2), number of day and night periods (P), and number of sampling blocks (n) represented by each histogram category (C) is shown. The histogram at the bottom of each set shows the distribution of all block medians from all cruises.

RELATION OF RANGE TO MEDIAN IN SAMPLING BLOCKS

Within block variation is indicated for each species group and for all species groups together by regression $\log R = a + b \log M$ (Table 4), where R = block range and M = block median. The slopes, b, for the five equations do not differ significantly from each other ($p = 0.05$). All are significantly greater than 0 ($p = 0.01$), and all but that for small copepods are significantly less than 1.0 ($p = 0.05$). The intercepts are all significantly greater than 0, again except for the small copepods, but they do not differ significantly from each other ($p = 0.05$). In view of the similarities, the regression for all groups combined (Figure 5) is a satisfactory description of the average relation of block range to block median for each of the species groups.

TABLE 4.—Estimated coefficients for regressions of log block range on log block median for each species group and all species groups combined.[1]

Species group	N	a	b	s	r
Small copepods	87	−.18	.95**	.40	.53**
Large copepods	81	.51	.72**	.36	.74**
Euphausiids	79	.38	.72**	.30	.79**
Chaetognaths	86	.26	.87**	.33	.82**
Combined	333	.35	.80**	.35	.93**

[1] N = number of sampling blocks; a = intercept; b = slope; s = standard deviation about the line; r = correlation coefficient.
** $p = 0.01$.

The regression for all groups combined indicates that average block range increases with block median but not proportionately. The expected ranges for different medians are:

Median	1	10	50	100	1,000	10,000
Range	2	14	51	89	562	3,548

Thus range will tend to be greater than the median when the latter is below 50, but appreciably less than the median when the median is above 100. Small copepods are the only group with consistently high medians, and they also have the greatest standard deviation about the line. This suggests that, while the ratio of range to median is lower, on the average, for this group, it also tends to be more variable than for the other groups.

The relation of block range to the total variation within the area is suggested by the magnitude of block ranges relative to the class intervals of the frequency distributions shown earlier (Table 5). In the case of small copepods, block ranges only slightly exceed the size of class intervals for the two highest classes in the distribution. For the other three species groups, block ranges exceed the class intervals for all but the lowest class. Ranges tend to be spread over three or more class intervals for the upper halves of the euphausiid and chaetognath distributions. Even though the highest classes tend to be rare, it can be seen that ranges extending over two or more class intervals would not be uncommon for large copepods, euphausiids, and chaetognaths.

TABLE 5.—The number of class intervals encompassed by the range for the midpoint of each class interval in the frequency distribution for each species group. The 0-1 class is excluded and other classes are numbered consecutively.

Frequency class	Small copepods	Large copepods	Euphausiids	Chaetognaths
1	0.1	0.6	0.7	0.6
2	0.3	1.4	1.6	1.4
3	0.9	2.1	2.4	2.1
4	1.2	2.8	3.2	2.8
5	1.5	3.4	3.9	3.4
6	--	--	4.6	4.0
7	--	--	5.2	4.6

DIURNAL VARIATION

It is evident from the differences in day and night sets of data that density level near the surface is influenced by diurnal vertical movements, particularly for the larger crustacean groups. The pattern of change is indicated for each of the species groups by medians for 4-hr time intervals (Figure 6). A sequence of change is most apparent for the large copepods and euphausiids, the lowest medians occurring between 1000 and 1400 hr and the highest between 1800 and 0200 hr. The increase in the evening appears to be more rapid than the decrease in the morning for both groups.

The small copepods show a pattern similar to that for large copepods, but much weaker. The highest time interval median is almost 10 times the lowest for large copepods but less than 2 times the lowest for small copepods.

Chaetognaths show only slight evidence of diurnal change. The peak between 0600 and

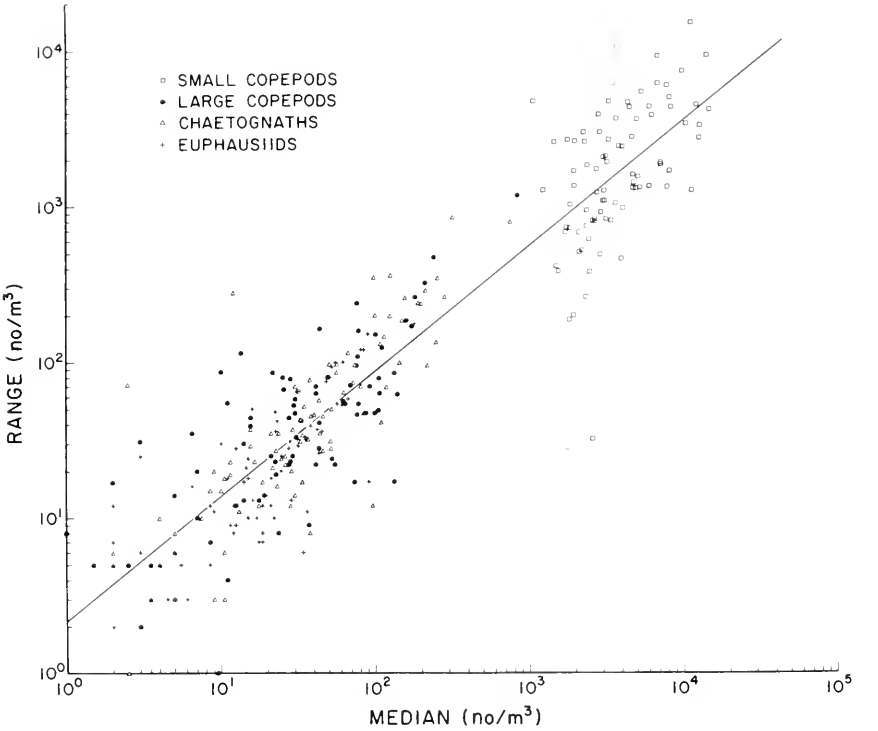

FIGURE 5.—The relation of sampling block range to sampling block median for the four species groups combined.

1000, which is obviously responsible for the generally higher day-period densities shown elsewhere, may indicate an upward and a downward movement in the morning.

The spread of block medians tended to be associated with the time-interval medians for euphausiids but not for the other species groups. The euphausiids showed both high and low block medians at night but only low medians during the middle of the day. Large copepods showed a similar distribution except that three of the highest four block medians in the series occurred between 1000 and 1800 hr. Small copepods and chaetognaths showed high and low medians in all time periods.

CORRELATIONS BETWEEN DENSITIES OF THE FOUR SPECIES GROUPS

The data were examined for association between the densities of species groups over the area by calculating rank-difference correlation coefficients (Tate and Clelland, 1957) for the nighttime block medians of each cruise (Table 6). Daytime blocks were excluded to reduce the component of correlation that would result from

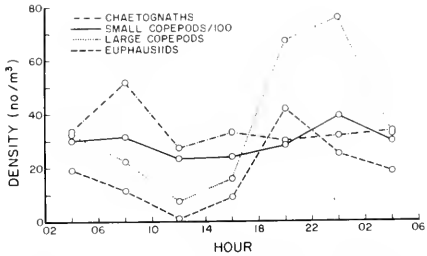

FIGURE 6.—The relation of median density to time of day for four species groups. The points are medians for block densities in 4-hr intervals. The small copepod medians were divided by 100 to put them on the same scale as the others.

TABLE 6.—Rank difference correlation coefficients for median block densities of five species groups for the night periods of five cruises.

	Cruise				
	1	2	3	4	5
Small copepods : large copepods	−.45	.31	.25	.71	.20
Small copepods : euphausiids	−.09	−.20	.59*	.14	.87**
Small copepods : chaetognaths	.26	−.36	−.43	−.42	.30
Large copepods : euphausiids	.10	.04	.71*	.14	.29
Large copepods : chaetognaths	.02	.29	.20	.21	.73*
Euphausiids : chaetognaths	.42	.12	−.23	.00	.53
Number of night blocks	10	10	10	7	9

* $p = 0.05$
** $p = 0.01$.

the parallel patterns of diurnal change in the crustacean groups.

The coefficients for each of the six species group combinations varied widely among the five cruises, with only four of the entire 30 coefficients indicating significant correlations. It appears that, while occasional correlations can be expected to occur over the area, consistent trends of association in density do not occur among these four species groups near the surface at night.

DRY WEIGHT VARIATION

Dry weight concentrations are summarized by cruise period in Table 7 and by weight class for all cruises in Figure 7. Low and high values occur both night and day, but there is clearly a shift to higher values at night.

The sample concentrations may underestimate the true concentrations by as much as 15 or 20%. Ahlstrom and Thrailkill (1963) showed that for copepods dry weight decreased about 15% after Formalin preservation. Lasker (1966) showed that dry weight of euphausiids was about 35%

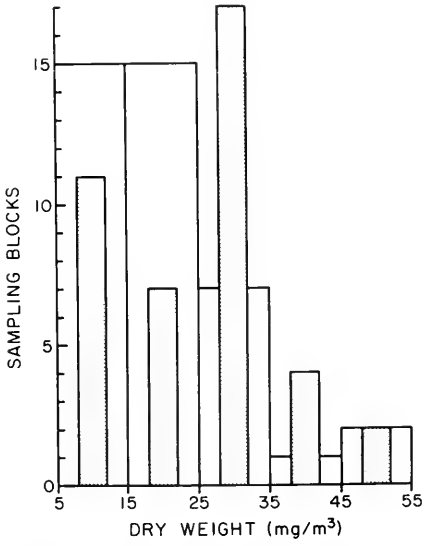

FIGURE 7.—Dry weight frequency distribution of all sampling blocks. The wide bars show day frequencies and the narrow bars night frequencies.

TABLE 7.—Summary of sample dry weight concentration (mg/m³) by cruise and day (D) or night (N) period.

	1D	1N	2D	2N	3D	3N	4D	4N	5D	5N
Mean	13.04	20.87	18.02	22.24	22.94	34.01	22.67	24.51	18.44	25.58
Median	15.00	18.32	14.51	21.03	22.76	31.80	15.24	24.72	15.27	23.23
Minimum	5.81	9.36	5.54	13.54	14.17	25.79	7.02	9.68	8.46	9.54
Maximum	20.85	34.00	43.74	30.57	30.42	49.59	56.90	34.62	32.11	52.42
Number blocks	7	9	6	7	7	9	11	7	9	9

lower for preserved than for fresh material. These groups were prominent in the pump samples, and dry weight determinations were made after long Formalin preservation. The pump samples undoubtedly contained a higher proportion of small copepod forms than the net samples of Ahlstrom and Thrailkill (1963), and it is possible that dry weight loss in Formalin is less for the smaller than for the larger individuals of this group.

As a basis for estimating the contributions of different species groups to dry weight concentration, dry weight factors were determined for large copepods, euphausiids, and chaetognaths by sorting known numbers of each from a few representative samples for drying and weighing. The resulting values are given in Table 8. The factor given for small copepods, which would have been difficult to separate in sufficient numbers and purity for a direct determination, was inferred from data given by Marshall and Orr (1955) for *Calanus finmarchicus* and *C. helgolandicus* in eastern Atlantic waters. They showed that *Calanus* stage V, at an average length of 2.5 mm, have a dry weight of about 300 mg 1000 organisms, and that according to Bogorov (1933) one stage V organism is equal in dry weight to two stage IV, 11 stage III, 42 stage II, and 60 stage I organisms. The average lengths of these stages are given as 2.1 mm, 1.65 mm, and 0.94 mm, and the average length of nauplii is given as 0.585 mm, which is the same as that of small copepods in the present study. Large copepods from the present study show an average length intermediate between those given for stages IV and III above, suggesting a ratio of six large copepods to one stage V, or a dry weight of 50 mg 1000 organisms, which is very close to the actual determinations. No dry weight equivalent is given by Bogorov for nauplii, but extrapolation of his series against average lengths suggests that 120 nauplii per stage V *Calanus*, or 2.5 mg 1000 nauplii, would be a conservative estimate.

TABLE 8.—Dry weight (DW) and ash weight (AW) determinations (mg/1000 organisms) for species groups in selected samples.

	DW	AW	AW/DW
	mg/1000	*mg/1000*	*%*
Small copepods	12.5	--	--
Large copepods:			
Day	54.64	3.48	6.36
Night	49.88	2.94	5.89
Euphausiids			
Day	42.03	3.29	7.82
Night	293.26	14.51	4.95
Chaetognaths	23.5	1.30	5.51

[1] Not determined by direct [illegible] (see text).

Dry weight concentrations were calculated for each species group in each block from the dry weight factors and from the medians of numerical estimates for the blocks. The values for species groups were summed to produce a "calculated" dry weight concentration for each block. These are compared to the measured dry weight concentrations in Figure 8. All the data together tend to cluster around the line of equal value (slope 1.0), and each of the different

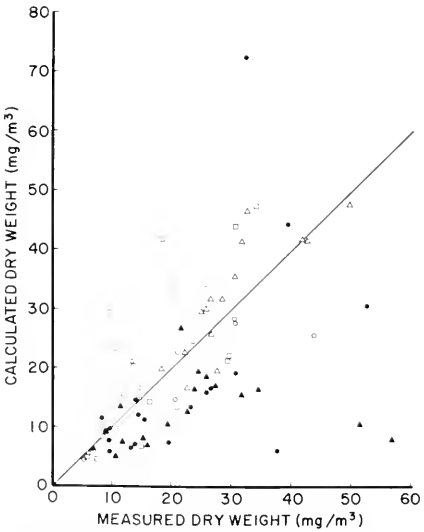

FIGURE 8.—The relation of calculated to measured dry weight concentration for all sampling blocks. Calculated dry weights were derived from species group density estimates and dry weight factors (Table 6). The line indicates equality of the two scales. ○ Cruise 1; □ Cruise 2; △ Cruise 3; ▲ Cruise 4; ● Cruise 5.

cruise sets has slopes similar to the line. Variation is wide, but there are only four serious discrepancies. Whatever the reasons for these discrepancies, the four blocks were excluded from calculation of the relative contributions of species groups to dry weight concentrations for day and night cruise periods.

Table 9 shows the averages of dry weights calculated for each species group for each cruise period, and for all cruise periods pooled by day and night. "Calculated" sample concentrations, obtained by summing the values for the four species groups, are compared with the average measured concentrations for the cruise periods in the last two columns. Though the calculated concentrations are lower than the measured concentrations for all day periods, and higher than the measured concentrations for three of the night periods, the two sets show reasonably good agreement, as do the day and night averages for all cruises together.

The measured sample concentrations for all cruises pooled suggest that, on the average, dry weight was 31% higher at night than during the day. The calculated concentrations suggest that it was 48% higher. Calculated values for the species groups show that the dry weight increase at night is largely attributable to increases in the euphausiid group, with lesser increases in the large copepods and also the small copepods. Euphausiids were responsible for more than one-third of dry weight concentration at night, on the average, and small copepods for less than half of it. During the day, on the other hand, small copepods accounted for about three-fourths of dry weight concentration, with most of the remainder divided between large copepods and chaetognaths.

VARIATION WITH TEMPERATURE AND DISTANCE FROM LAND

The data for each of the four species groups, and for dry weight, were examined for possible relationships with the independent variables, temperature, miles from nearest land (including islands), and miles from nearest point on the mainland. Regressions were in the form log $Y = a + bX$, where X = the independent variable and Y = the dependent variable. Small copepods, large copepods, and dry weight showed significant trends with temperature, and chaetognaths showed significant trends with distance from land (Table 10). For euphausiids, night values alone, as well as day and night values together, were tested but neither demonstrated significant trends.

The two copepod groups and dry weight all show an inverse relation with temperature, but in all cases the trends are largely attributable to changes occurring in 1961, as a comparison of nighttime cruise medians with average cruise temperature shows (Table 11). The decline in

TABLE 9.—Calculated average dry weight fractions (mg/m³) and percentages for species groups by cruise and day (D) or night (N) period.

	Small copepods		Large copepods		Euphausiids		Chaetognaths		Average sample dry weight	
	mg/m³	%	mg/m³	%	mg/m³	%	mg/m³	%	Calculated mg/m³	Measured mg/m³
1D	6.46	*70.9*	0.26	*2.9*	0.26	*2.9*	2.13	*23.4*	9.11	13.04
1N	7.70	*26.1*	3.49	*11.8*	14.88	*50.5*	3.41	*11.6*	29.48	20.87
2D	8.54	*61.4*	2.18	*15.7*	0.36	*2.6*	2.84	*20.4*	13.92	18.02
2N	9.29	*35.7*	4.08	*15.7*	11.83	*45.5*	0.79	*3.0*	25.99	22.24
3D	20.07	*88.3*	0.73	*3.2*	0.63	*2.8*	1.29	*5.7*	22.72	22.94
3N	25.74	*68.7*	3.70	*9.9*	6.99	*18.7*	1.03	*2.7*	37.46	34.01
4D	6.05	*62.6*	0.97	*10.0*	0.35	*3.6*	2.30	*23.8*	9.67	15.66
4N	8.88	*51.0*	1.50	*8.6*	4.95	*28.4*	2.07	*11.9*	17.40	24.51
5D	7.56	*66.4*	2.93	*25.7*	0.28	*2.5*	0.61	*5.4*	11.38	16.72
5N	8.52	*47.7*	2.09	*11.7*	6.86	*38.4*	0.41	*2.3*	17.88	24.09
Average D	9.74	*72.9*	1.41	*10.6*	0.38	*2.8*	1.83	*13.7*	13.36	17.28
Average N	12.03	*46.9*	2.97	*11.6*	9.10	*35.5*	1.54	*6.0*	25.64	25.14

TABLE 10.—Regressions of median block densities and dry weight concentrations on temperature and distance from land.[1]

Regression	N	a	b	s	r
Small copepods on temperature	83	5.38	—.105*	.23	.54**
Large copepods on temperature	78	3.23	—.101*	.52	26*
Dry weight on temperature	78	2.46	—.067**	.22	.40**
Chaetognaths on distance to mainland	85	1.99	—.013**	.53	31**
Chaetognaths on distance to nearest land	85	2.02	—.020*	54	.22*

[1] N = number of sampling blocks, a = intercept, b = slope; s = standard deviation about the line, r = correlation coefficient.
* $p = 0.05$
** $p = 0.01$.

TABLE 11.—Average temperatures, median copepod densities, and median dry weight concentrations for the night periods of each cruise.

Cruise date	Temperature	Small copepods	Large copepods	Dry weight
	°C	no./m³	no./m³	mg/m³
9/27/61	18.9	2,542	42	18
10/10/61	18.2	3,325	101	21
11/17/61	15.9	10,281	77	32
10/ 2/62	18.6	3,504	21	25
11/20/62	16.1	3,750	30	23

water temperature was approximately the same in both years. The copepod values and dry weight consequently show a strong inverse relation with temperature and date for 1961 but not for 1962.

The chaetognaths show inverse trends with distance from the mainland and from nearest land, but the former is the more significant of the two. The relationship with distance to nearest land includes many of the distance measurements to the mainland, of course, and it is possible that these are largely responsible for the significant relationship with distance to nearest land. The geographical distribution of all chaetognath block medians (Figure 9) shows that distance from the mainland is the more pertinent independent variable. Low densities occurred at all distances beyond 7 miles, whereas the highest densities did not occur farther offshore than 11 miles, with a single exception. It can be seen that density was far more variable near the mainland than offshore.

DISCUSSION

The food potential of plankton for pelagic fishes depends on the relation between the average density of some or all species groups over an area and the rate at which the fishes can feed on these species groups. Since median density of the more common species groups varies widely within the space of a few months for the area surveyed in this study, it is probable that food potential of near-surface plankton off southern California fluctuates appreciably within short time intervals. However, there were marked small-scale variations in the distributions of densities associated with the general area levels, and the general level would not be an appropriate index of food potential if fishes tend to orient to small-scale features of distribution.

Although the association of range with median for sampling blocks of 51.8 km² demonstrates that densities vary, sometimes widely, within the blocks, the medians of blocks of this or some similar size probably constitute a scale of sufficient resolution for assessing the food potential of plankton over a large area. Maximum values within the blocks are not likely to be impressively greater than the median. If the median of large copepods is 175 m³, the block can be expected to have, on the average, a maximum density of 245 m³ representing an area of 2.6 km² (1 square mile) within the block. If the median of euphausiids is 90 /m³, the block can be expected to have a maximum density of 130 m³ for 2.6 km². Blocks with such medians are usually rare, and the medians, as well as the maximum values, are likely to be considerably higher than the densities in most blocks in the area. The medians would slightly underestimate the food potential of such blocks only if plankton feeding fishes tend to orient to the highest densities within the space of 20 square miles.

The distributions of sampling block medians, which were skewed unless general area level was very high, suggest that even under the poorest general conditions relatively high densities of organisms are likely to exist in some small portion of the survey area. Small copepods, for example, showed a few occurrences of blocks with medians above 6000 m³, and one

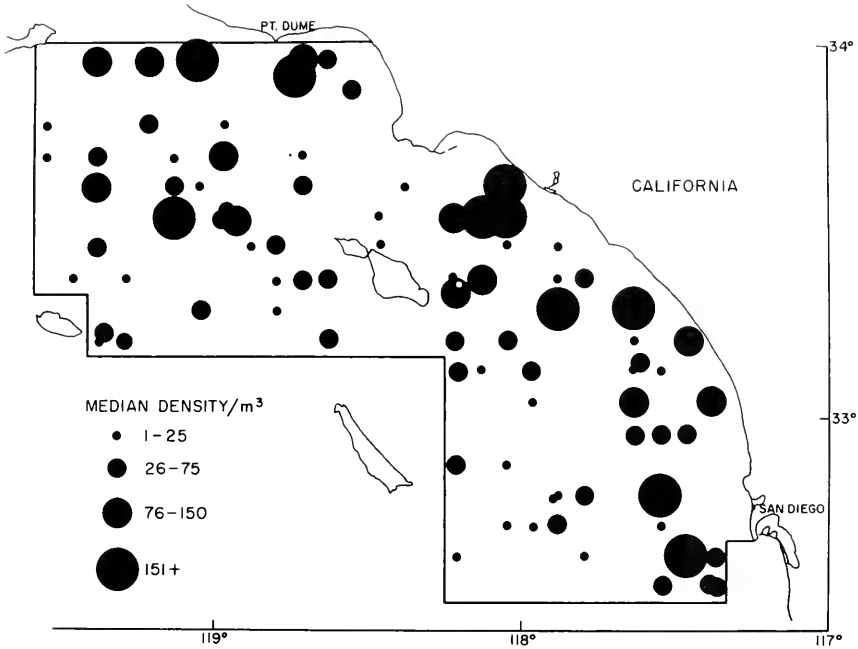

FIGURE 9.—Geographical distribution of chaetognath medians for all blocks on all cruises.

with a median above 9000 /m³, when the general area median was about 3000 /m³. Large copepods showed a few occurrences of blocks with medians well above 200 when the general area median was 50 /m³ or less. Euphausiids showed occurrences of blocks with medians above 75 /m³ when the general area median was between 25 and 40 /m³. It seems probable, in other words, that high crustacean densities are always present somewhere in the area. At the lower general levels they would be scarce but perhaps as much as three times higher than densities over most of the area.

The data indicate that higher densities of chaetognaths are most likely to occur near the mainland, but they failed to demonstrate such trends for the three crustacean groups. They indicate only that the crustaceans, and dry weight, may sometimes be at higher levels where and when temperatures are relatively low. Regressions with temperature show, for example, that the density of small copepods was 6400 /m³ in 15° C water, on the average, as compared with 2400 /m³ in 19° C water, and that dry weight concentration was 29 mg /m³ in 15° C water, but only 15 mg /m³ in 19° C water. Since variation associated with these trends is wide, it can only be concluded that water of low temperature may sometimes, though not always, contain much higher standing crops of zooplankton than are likely to be found in warmer water in the survey area.

Dry weight factors were determined for the different species groups because it is impossible

to estimate the relative nutritional value of such groups in the plankton on the basis of organism counts alone. In general, the near-surface zooplankton had a dry weight equivalent of about 25 mg/m³ at night, which was approximately 30% greater than the average daytime level. Small copepods were the dominant fraction during the day and increased only slightly at night. Most of the nighttime increase in dry weight is attributable to the appearance of euphausiids, which were estimated to have a dry weight equivalent of 9 mg/m³, on the average, as compared with less than 1 mg/m³ during the day. There was some deviation from this general pattern among the five cruises. Differences between and within sampling blocks were not described in terms of dry weight. The general differences are enough to show that the nutritional potential of the plankton, in terms of dry weight for any set of samples, would depend on the extent to which fishes do or do not feed selectively.

However they are interpreted, it must be noted that the dry weight equivalents of the samples taken in this survey, aside from the possible loss of weight in Formalin preservation, may not represent the whole of the biomass utilized by some plankton feeding fishes near the surface. They contained almost no zooplankters smaller than 0.2 mm in length and relatively little phytoplankton. The comparison in Table 12 indicates that such smaller organisms may constitute a considerable fraction of the biomass.

Beers and Stewart (1967) sampled the euphotic zone with a towed pump on a line of stations off San Diego. Water was strained successively through 202-, 103-, and 35-μ cloths to estimate quantities below each cloth. Leong and O'Connell (1969) estimated from the resulting data that phytoplankton and zooplankton passing through the 103-μ cloth represented average dry weight concentrations of 25 and 3 mg/m³. The 103-μ cloth was approximately the same mesh size as the filtering screens used in the present survey. The material retained between the 103- and 202-μ cloths of Beers and Stewart is here judged to approximate the size range, 0.2 to 1.0 mm, and the numerical estimate for their innermost station, when converted to dry weight by the constant, 2.5 mg/1000 organisms, yields a concentration similar to the daytime average for crustaceans in this size range in the present surveys.

It appears that material smaller than that collected in the present survey might represent a dry weight approximately two to three times as great as that that was collected. Adjustment for the smaller organisms in questions of nutritional potential would depend on selectivity in the feeding of various fishes.

It is difficult to judge whether the dry weight values attributed to organisms larger than 1 mm in the above comparison fully represent the biomass of larger plankton organisms near the surface that can be utilized by plankton feeding fishes. Such fishes probably take crustaceans larger than collected by the pump when opportunity arises. It can only be restated that euphausiids larger than those sampled are relatively rare close to the surface. Five of the 1-m-mouth-opening-net tows taken by Ahlstrom and Thrailkill (1963) through the upper 100 m or so were composed largely of crustacean material. From the data given they were estimated to represent dry weight concentrations averaging 3.5 mg/m³. The figure is in the range of day and night concentrations for the pump samples, but the comparison is uncertain because of differences in the location as well as the depth of sampling.

The above discussion implies that estimating the food potential of plankton for fishes must depend as much on information concerning the feeding behavior of the fishes as on information concerning the abundance and variability of the plankton. The data given here on plankton variability are intended as a basis for interpreting hypotheses that may arise from laboratory or field studies of feeding behavior.

TABLE 12.—Comparison of dry weight values (mg/m³) for different length ranges of planktonic organisms in two towed pump studies.

	Period	Group	Length range (mm)		
			<0.2	0.2–1.0	>1.0
Beers and Stewart (1967)	Day only	Phytoplankton	25.1	0	0
		Zooplankton	2.9	8.9	0
Present survey	Day	Crustacean	0	9.7	1.8
	Night	Crustacean	0	12.0	12.1

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NOTE

PREDATION ON JUVENILE PACIFIC SALMON BY A MARINE ISOPOD *Rocinela belliceps pugettensis* (CRUSTACEA, ISOPODA)

Observations were made of predation by a marine isopod on both captive and wild Pacific salmon. Pacific salmon are known to be hosts to a number of ectoparasites, especially the Copepoda. However, we have been unable to find any reported incidents of predation or parasitism of Pacific salmon by the Isopoda.

In July 1969, we began a series of experiments in Puget Sound to evaluate the feasibility of saltwater rearing of Pacific salmon within floating pens. Stocks of young salmon were weighed and measured several times during the year at the Manchester, Wash., experimental station of the National Marine Fisheries Service and routinely examined for ectoparasites visible to the naked eye. A large Branchiuran parasite, *Argulus* sp., was found on a 600-g chinook salmon (*Oncorhynchus tshawytscha*); the Branchiuran was removed from the fish and kept for study. Later, another Branchiuran was found in the same body position on this fish. There were no other visible ectoparasites.

In March 1970, we began collecting zooplankton to provide live food for both salmon fry and newly metamorphosed flatfish. Collections were made during the night; a surface light was used to attract the plankton and an airlift pump to draw them into a net suspended at the sea surface.

The collected organisms were poured into a tank containing over 1000 young pink salmon (*O. gorbuscha*), 30 to 45 mm long. Among the unsorted plankton were 8 or 10 isopods of the same species. The pink salmon fed vigorously on large numbers of amphipods in the tank, whereas the isopods alternately rested on the sides of the tank and swam about in random patterns. One isopod quickly attached itself to a young pink salmon. Within minutes, two more of the young salmon were similarly attacked. The fish became distressed, swam erratically about the tank, and drifted listlessly to the bottom, where they died within a few minutes after settling. The point of attack by the isopod on each young salmon was lateral, just above or slightly posterior to the pelvic fin. Small, but deep wounds that penetrated the body wall were found on each affected fish. The gut of each of the isopods removed from the dead fish contained blood.

One of the isopods was placed in another tank containing several dozen coho salmon (*O. kisutch*), slightly larger than the pinks (100-140 mm long). An hour later, the isopod was found firmly attached to the head of one fish, immediately posterior to and between the eyes.

Isopods are now removed before any plankton are fed to our fish. If we should overlook even the smallest of these isopods, we can expect either death or injury to some fish.

We examined several specimens of the hundreds of isopods we have collected and have identified them as *Rocinela belliceps pugettensis* (Stimpson), a subspecies of *Rocinela belliceps*. *R. belliceps* is widespread in northern coastal areas of the North Pacific Ocean, from the Bering Strait southward along the North American coast to California and southward along the Asian coast to Korea (Pavlovskii, 1955). Hatch (1947) feels that most of the specimens in Puget Sound are the subspecies, *pugettensis*. He assigns subspecific rank on the basis of the number of spines on the propodite of the prehensile legs —six spines on the propodite of *pugettensis*, whereas *belliceps* has three to four. A zone of mixed populations of *R. belliceps belliceps* and *R. belliceps pugettensis* exists near the entrance to Hood Canal, just inside the Strait of Juan de Fuca. All of the specimens we examined from our collections at the Manchester station (which is on the western side of central Puget Sound) had six spines on the propodite of the prehensile legs.

Rocinela sp. belongs to the family Aegidae. Members of this family are widely recorded ectoparasites of fishes. The first three pairs of walking legs are prehensile and are used effectively for attachment.

We originally felt that the attacks on our salmon fry and fingerlings by *R. belliceps pugettensis* might have been the result of confinement and would not be likely to occur with such frequency in open waters. However, on at least three occasions, we have observed wild chum salmon (*O. keta*) and pink salmon fry and fingerlings attacked by *R. belliceps pugettensis*. These attacks occurred at night, under a light, that probably attracted larger numbers of fish and isopods than would normally occur in open waters. In each instance, an isopod attached itself at a point just posterior to the dorsal fin and on, or slightly below, the lateral line. Afflicted fish could not maintain a normal swimming position in a school and darted about in erratic patterns. On one occasion, an afflicted fish was seen to leave the school and disappear. Even if a wound inflicted by an isopod were not fatal, it is possible that the erratic behavior of a fish trying to dislodge the parasite might attract predators.

On another occasion, in one of our large floating pens, we found a juvenile coho salmon with *R. belliceps pugettensis* attached anterior to the dorsal fin and just above the lateral line (Figure 1). This pen was not near our night light and there was a large amount of free space compared with our tanks or cages.

Also, in a cage that had a submerged light to attract plankton, we found *R. belliceps pugettensis* on an immature coho salmon weighing about 200 g. The fish appeared to be in some distress, but perhaps because of its size, the wound was not fatal.

Arai (1969) reported on 68 taxa of parasites recovered from 61 species of fish in British Columbia, but these did not include *R. belliceps belliceps*. Arai's collections were made by seine and trawl; we found that *R. belliceps pugettensis* will release its grasp if the host is forced into a restrictive net. Hatch (1947) states that *R. belliceps belliceps* is found in 9 to 1250 m of water and that it is an ectoparasite of cod, sculpin, halibut, skate, and other bottom fish. At Manchester, where our observations were made, the water depth is 9 to 13 m. The abundance of *R. belliceps pugettensis* varies between seasons, with the greatest abundance from April through August.

Although we have no way to judge the extent of natural predation of Pacific salmon by *R. belliceps belliceps* or *R. belliceps pugettensis* in the wild, we think that, especially under the confined conditions of pen rearing, the fry and juveniles of Pacific salmon should be included as possible prey of *R. belliceps pugettensis* and probably *R. belliceps belliceps*.

The present instance clearly points up the possible misapplication of the term "parasitism" in certain cases of specialized predation. The isopod *R. belliceps pugettensis* is not a permanent symbiont of a fish and is thus not properly referred to as a parasite.

FIGURE 1.—*Rocinela belliceps pugettensis* on a juvenile coho salmon in one of the large growing pens. This specimen was anterior to the dorsal fin, whereas most of the others were attached in a posterior position. Note the blood-filled gut of the isopod.

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MEASUREMENTS OF FISH TARGET STRENGTH: A REVIEW

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ABSTRACT

The concept of target strength and its application to the quantitative assessment of fishery resources are discussed. Methods of determining the echo characteristics of fish are reviewed and a number of results presented. Among the more important of these results are: (1) practically every case of interest to the fishing industry is in an acoustic region in which the target strength varies widely with fish size and aspect and acoustic frequency, (2) the major contributors to target strength in this region have been determined to be the swim bladder, flesh, and skeleton, and (3) the average maximum side-aspect and dorsal-aspect target strength of an individual fish have been determined for this region.

Quantitative assessment of fishery resources is a difficult task, and many groups have turned to acoustic techniques to conduct assessment surveys. Present acoustic techniques can give an estimate of the number and dimensions of fish schools in a geographic area and, by estimating the density of the fish in a school, the number of individual fish in the area can be approximated. Measurements of fish target strength are being made by various investigators in an effort to enable the direct acoustic estimation of the number and size of individuals in a school and to enable the direct identification of those individuals by acoustic methods. This paper discusses the concept of target strength and its application to the quantification and or identification of fish schools, reviews target strength measurement techniques, and discusses some results which have been obtained utilizing these techniques.

TARGET STRENGTH

Active sonars project acoustic energy into the water in an effort to detect objects by the echoes they return, the intensity of the echo depending on the proportion of the sound reflected back to the receiver. The target strength of the echo-producing object is a quantitative measure of its reflecting characteristics and is defined as

$$T = 10 \log \left(\frac{I_1}{I_0} \right), \qquad (1)$$

where I_0 is the intensity of the sound striking the target and I_1 is the intensity of the reflected sound measured at 1 m from the acoustic center of the target. If I_r is the intensity of the reflected sound measured at some distance r from the target, then, assuming that the sound spreads spherically, and there are no losses, I_r will be directly proportional to I_0:

$$I_r = \left(\frac{\sigma}{4\pi} \right) \frac{I_0}{r^2}. \qquad (2)$$

σ is defined as the acoustic cross-section of the target, and $4\pi r^2$ is the spherical surface area through which all the incident energy is reflected. σ depends on the size, shape, and orientation of the target, and, in general, will vary with the angle between the incident direction and the direction of the receiver. In all present fisheries work, this angle is zero and therefore in this paper σ will be the acoustic cross-section of a target for the case in which the source and receiver are located at the same point.

By letting r in equation (2) be equal to 1 m and combining equations (1) and (2),

$$T = 10 \log \left(\frac{\sigma}{4\pi} \right). \qquad (3)$$

Now if I_s is the intensity of the projected sound 1 m from the source,

$$I_0 = \frac{I_s}{r^2} \qquad (4)$$

and therefore,

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$$I_r = \left(\frac{\sigma}{4\pi}\right) \frac{I_s}{r^4}, \quad (5)$$

or in logarithmic form,

$$10 \log I_r = 10 \log \left(\frac{\sigma}{4\pi}\right) + 10 \log I_s - 40 \log r. \quad (6)$$

Defining the echo level (E) as 10 log I_r and the source level (S) as 10 log I_s, and rearranging,

$$T = E - S + 40 \log r. \quad (7)$$

Equation (7) can be used to compute target strength in an ideal medium. In a real medium, however, the actual transmission loss will include effects due to absorption, scattering, refraction, and the boundaries. Hence, in a real medium

$$T = E - S + 2H \quad (8)$$

where H is the one way transmission loss. H takes into account spreading loss, absorption, and any anomalies. In actual practice H cannot always be reliably predicted and must be measured unless the ranges involved are small. Equation (8) is known as the active sonar equation and is always used in the computation of target strength since it involves only directly measurable quantities—echo level, source level, and transmission loss.

It is impossible for more sound to be reflected from a target than is incident upon it, and it is therefore seemingly impossible for any object to have a positive target strength, yet many large targets do. This is a consequence of the reference distance being 1 m, and the measurements being made at greater distances, with spherical spreading assumed in order to calculate the target strength. However, the spreading loss very close to a target is less than the spherical spreading loss which is assumed, and hence positive target strengths can be obtained for large targets.

The importance of the target strength of a potential target is obvious from the sonar equation (equation (8)). The maximum range at which a target can be detected in any given environment depends on its target strength and the characteristics of the transmitting and receiving systems. Therefore, an estimate of target strength is essential to the effective design and operation of any active sonar system.

The quantification of a fish school, knowing its target strength, is possible because the target strength of a school depends on the average size, number, distribution, and aspect of the individuals in the school. In order to quantify fish schools using target strength information, it is first necessary to determine the size and number of individuals required to produce a given target strength. The initial step in this process is the determination of the target strength of an individual fish. The application of this knowledge to studies on the acoustic interactions of arrays of scatterers will eventually produce accurate predictions of school target strength. The great majority of work done up to this time has been on individual fish, and quite a bit more must be done before this initial step is completed. Definitive work on the quantification and identification of fish schools utilizing target strength information awaits completion of this step.

Reflection of sound from an object in water occurs when the object has an acoustic impedance which differs from that of the water. Acoustic impedance is defined as the product of the density (ρ) of a substance and the velocity of sound (c) in that substance. The proportion of sound reflected from or transmitted into an object in water depends on the magnitude of the impedance mismatch between the object and the water. The simplest case of reflection occurs when a plane wave is normally incident upon a plane boundary between two semi-infinite media. The pressure amplitude reflection coefficient is defined as the ratio of the reflected pressure amplitude to the incident pressure amplitude, and for this case it is found to be $\frac{\rho_2 c_2 - \rho_1 c_1}{\rho_2 c_2 + \rho_1 c_1}$, where $\rho_1 c_1$ is the impedance of the medium in which the incident wave is traveling and $\rho_2 c_2$ is the impedance of the medium upon which the wave is incident. If the second medium is reduced to finite thickness and a third medium is placed behind it (the third medium may or may not be the same as the first), the problem becomes slightly more complicated. When the incident wave arrives at the first boundary, some energy

is reflected and some is transmitted. After this transmitted portion reaches the second boundary, some of the energy is transmitted into the third medium and some is reflected back into the second medium, where it is again partially reflected from the first boundary. This process continues until a steady state is reached. The solution of this problem is relatively easy, but it is interesting because the resultant amplitudes of the transmitted and reflected waves depend on the phases of the component waves. The component waves add vectorially and whether the amplitude of the initially reflected wave is increased or decreased depends on the thickness of the second medium and the wavelength of the incident wave.

The reflection of sound from an infinite plate poorly approximates the reflection from a fish, and it is useful to examine the reflection from a finite object such as a sphere. When the sphere is large compared to the acoustic wavelength, the echo originates from specular reflection, in which the part of the sphere near the point where the sound wave is normally incident produces a coherent reflected wave. When the size of the sphere is comparable to a wavelength, interference effects similar to those mentioned for the plate with finite thickness will cause the acoustic cross-section to vary. When the sphere is small compared to a wavelength, scattering takes place and the acoustic cross-section of a sphere of radius a is proportional to $a^6 \cdot \lambda^4$ where λ is the wavelength. This solution was obtained by Lord Rayleigh and hence this region is called the region of Rayleigh scattering.

For objects other than spheres, analysis becomes difficult, if not impossible. However, as long as the object is not highly compressible, the concept of the regions of Rayleigh scattering, interference effects, and geometric reflection is valid. For a fish, the distinctions between these regions becomes unclear because of the fish's internal structure. When the fish is very small compared to the acoustic wavelength, Rayleigh scattering can be expected. However, if the fish has a gas-filled swim bladder the gas bubble will resonate at some wavelength in this region, greatly increasing the target strength over that predicted by Rayleigh scattering. When the size of the fish is comparable to the wavelength, interferences will occur among the fish flesh and organs, the skeleton, the gas in the swim bladder, and the boundaries of the fish. When the fish is larger than the wavelength, the dimensions of many of these parts will be comparable to the wavelength and the region of interference effects will be greatly extended into what would be the region of geometric reflection for a homogeneous body.

Cushing et al. (1963) have assumed that the region of interference effects extends from $L/\lambda = 8$ to $L \lambda = 100$, where L is the fish length, and λ is the acoustic wavelength, and they suggest that for quantitative results this region should be avoided. Neglecting the fact that they have ignored the effects of swim bladder resonance, the limits they have placed on the interference region will now be examined. For a rigid sphere of radius a the limits of the interference region are approximately $1 \leqslant 2\pi a/\lambda \leqslant 10$, and for any other object these limits will probably be farther apart. Measurements on individual fish indicate that interference effects occur at values of $L \lambda = 0.7$ (Love, 1971) and this can be taken as a lower limit. (This is not to say that it is the lower limit, only that this is as low as measurements have been made.) Haslett (1962a) examined a small number of whiting to determine their "standard dimensions." He found that the diameter of the backbone was about 0.01 the length of the fish. Assuming that the backbone of a fish is the smallest part of a fish which contributes to its echo, this means that if interference effects occur in the backbone until its circumference is something near 10 times as large as the wavelength, as in the case for the sphere, then the upper limit of the interference zone for a fish will be at least $L/\lambda = 200$. Again, measurements have been made which indicate that the upper limit will be at least this high (Haslett, 1969). Therefore, it may be assumed that the limits of the interference region are at least $0.7 \leqslant L/\lambda \leqslant 200$.

If it is assumed that fish of interest to commercial fishermen range from 10 cm to 150 cm, and that fish-finding sonars have frequencies ranging from 10 kHz to 200 kHz, then the range of interest for fisheries applications will be $0.7 \leqslant L/\lambda \leqslant 200$, the limits set for the interference

region. Therefore, although it would be advantageous to avoid the interference region, it is apparent that this is the region in which the work must be done.

METHODS OF TARGET STRENGTH MEASUREMENT

Analytical methods are of limited value in this interference region and experimental methods must be utilized to obtain any valid answers. It is possible to conduct the needed experiments either at sea or in the laboratory, with each type of measurement having its limitations. Whether the measurements are made at sea or in the laboratory, target strength will be determined from the sonar equation, meaning that the source level of the transmitter, the sensitivity of the receiver, and the propagation loss must be known. The calibration of the transmitting and receiving systems is a standard procedure, but propagation loss is much more difficult to determine if long ranges are involved. One way to avoid the propagation loss problem is to make measurements at short ranges, and this is what is done in the laboratory. This, of course, is impossible with large targets. Another method is to use a reference target for which the target strength is known. In this method neither the transmitting nor receiving systems have to be calibrated and the propagation loss does not have to be measured because all echo levels are compared to the reference level. One of the best reference targets is a thin-walled air-filled rubber sphere, although for large targets buoyancy becomes a problem. Another good reference target, which can be used for large targets, is a tri-plane, three mutually perpendicular planes, for which it can be shown that any incident ray will be reflected in a direction exactly opposite to the incident direction. Hence a tri-plane acts as a single plane perpendicular to the incident rays and reflects a large, calculable percentage of the incident energy. It is possible to measure propagation loss directly and this is fairly simple if a transmitting and a receiving ship are utilized. Propagation loss measurements can also be made by placing a calibrated transponder in the vicinity of the target.

Along with the problem of accurately measuring propagation loss, there are other problems associated with target strength measurements at sea, the most critical of these being relative motion between the sonar beam and the fish target. Roll or pitch of the ship can be overcome by using a stabilized sonar beam, but drift can cause the axis of the beam to move off target. Care must also be taken so that the target support structure does not interfere with the measurements. Other problems that can arise are poor weather, high ambient noise levels, and extraneous targets swimming into the beam.

Of course, there are problems associated with laboratory measurements also, the chief one being the limitation on the size of the target. The fish must be placed at a range great enough to insure that the incident sound energy is approximately equal over the complete fish and to insure that the fish is not in the near-field of the transmitter nor the receiver in the near-field of the fish. However, the range must not be so great that reflections from the boundaries or fish support interfere with the measurements. Nevertheless, by judicious choice of measurement range and by using short pulse lengths, unambiguous work can be done in a laboratory tank. In order to obtain a true value of target strength the pulse lengths of the discrete frequency pulses most often utilized by fisheries sonars must be at least twice the length of the target in the direction of propagation, so that an echo can be obtained from all parts of the target simultaneously.

A typical block diagram of the electronics required for target strength measurements is shown in Figure 1. The transmitting system consists of a signal source of known frequency, a means to generate pulses, amplifiers, a transmitting transducer, a system to match the electrical impedances of the amplifier and transducer, and a means to measure the outgoing signal. The receiving system consists of a receiving transducer, amplifiers, a means to gate out unwanted echoes, possibly a filter, and a means to measure the received signal. The electronic system is basically the same whether it is used in the laboratory or at sea.

If all fish were composed of the same homoge-

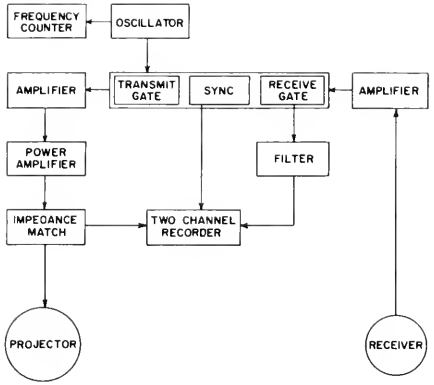

FIGURE 1.—Typical target strength measurement system electronics.

neous material and had the same shape, it would take a comprehensive measurement program to determine the target strength of a fish at all aspects and frequencies because of the complex shape of the body. Since fish are definitely not homogeneous and different species of fish have different shapes and internal structures, the problem of determining the target strengths for all species becomes immense. Considering the differences among individuals of the same species due to age, sex, condition of health, etc., it is obvious that an experimental program to predict completely the target strength of any given fish is impossible. Since the complete determination of the target strength of all fish is impossible, the most that can be hoped for is that experimental techniques will eventually lead to empirical results which can be generalized to apply to any species of importance.

RESULTS OF MEASUREMENTS OF INDIVIDUAL FISH

Most of the early experiments conducted during the 1950's investigated a specific situation. For example, if a researcher had an echo sounder which operated at a given frequency, he would measure the dorsal-aspect target strengths of a number of fish of the commercial species found in his geographic area, in order to obtain an average dorsal-aspect target strength vs. fish size for those species. This information was valuable to anyone designing or using an echo sounder of the same frequency to find these species, but it was of minimal value to anyone else.

A second technique used is just an extension of the earlier technique. With it, different species of fish have been examined at many aspects and/or frequencies in attempts to determine how target strength varies with fish size, species, aspect, and frequency. Figure 2 shows some typical results of this technique. The results are from a live 21-cm black crappie which was rotated about its dorsiventral axis and insonified with frequencies of 30 kHz and 130 kHz. It is seen that the maximum target strength occurs very near the side aspect, where the insonified area is a maximum. At 130 kHz the number of lobes in the pattern is substantially greater, and

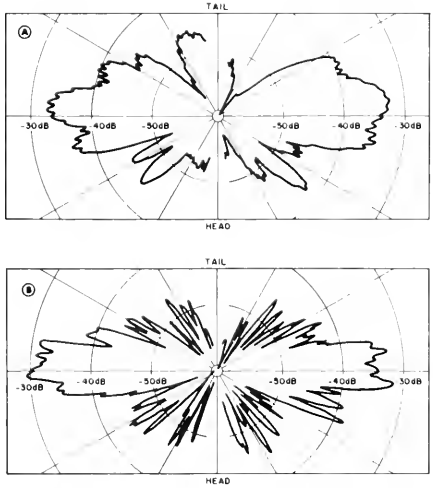

FIGURE 2.—Target strength of a 21-cm black crappie versus aspect. (a) 30 kHz. (b) 130 kHz. (From Love, 1969.)

the maximum target strength slightly greater than at 30 kHz. Although these measurements have produced some useful results on the general changes of target strength with fish size and frequency for aspects of special interest, little has been learned about the fine-scale changes or about the differences among species.

The use of a third technique permits investigation into the nature of the echo-formation process either by dissection or modeling. By dissection, researchers have discovered which parts of a fish are the major acoustic reflectors. By removing the swim bladders from a number of perch, Jones and Pearce (1958) determined that the gas-filled swim bladder accounts for approximately 50% of the dorsal and side-aspect target strengths of perch at $L\ \lambda = 4$. Hence, the swim bladder is an important contributor to target strength for L/λ values in the interference region. By systematically removing various parts of a skipjack tuna, Volberg (1963) found that appreciable echoes could be obtained from either the skeleton or a piece of flesh. Diercks and Goldsberry (1970) have indicated the possibility that scales may also be an important contributor to the target strength of a fish at certain frequencies. Unfortunately, they did not remove any scales and their hypothesis is based on considerations of the directivity of the scales as an array of scatterers.

An adjunct to the determination of the parts of the fish which are acoustically important is the determination of the acoustic impedance or reflection coefficient of these parts. The reflection coefficient is defined as it was previously for two semi-infinite media. The impedance of the gas in the swim bladder is readily determined, and the reflection coefficient for the swim bladder is approximately —1. Determination of the acoustic impedance of fish bone or flesh is difficult and care must be taken to insure valid measurements. Shishkova (1958) measured the density of and speed of sound in flesh from a few species of fish and determined the reflection coefficient in fresh water to be about 0.05. Haslett (1962b) used a different technique to indirectly measure the reflection coefficients of flesh and bone from haddock and cod. He found the reflection coefficient of flesh in fresh water to be about 0.05, in seawater to be about 0.02, and the reflection coefficient of bone to be about 0.25.

Using these values for the reflection coefficients and his "standard fish dimensions," Haslett (1962c, 1964) has modeled fish bodies, backbones, and swim bladders. Utilizing rubber ellipsoids to model the fleshy body of the fish, he found that the number of lobes obtained in polar plots for the ellipsoids and for actual fish agreed fairly well, that is, with less than 50% error, but that the target strengths obtained for the models were considerably lower than those obtained for the fish. Using rubber and plastic cylinders to model the backbone and copper cylinders to model the swim bladder, Haslett has examined variations in the target strength of these models as frequency, size, and aspect are varied. A brief summary of Haslett's work for side aspect is shown in Figure 3. Along with his data for the acoustic cross-sections of sticklebacks and guppies, approximations to the acoustic cross-sections of the swim bladder, body, and backbone are given. The various curves for each component were determined by Haslett (1965) using his reflection coefficients and the results of his modeling experiments and depend

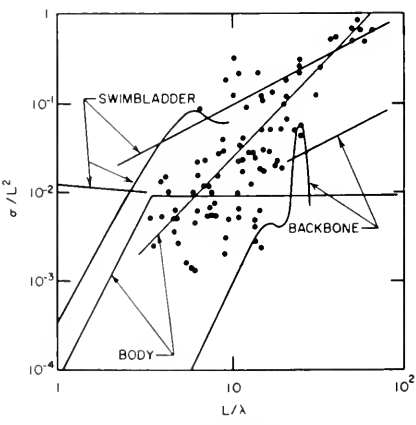

FIGURE 3.—Side-aspect acoustic cross-sections determined by Haslett.

on how the component is approximated and on the limits of that approximation. Hence the curves are a function of whether the swim bladder is approximated by a spherical bubble or a rigid cylinder; what the limits of the geometric and Rayleigh scattering regions are for the body and the backbone; and whether the body is approximated by an ellipsoid or a plane in the geometric region. These curves indicate that the swim bladder predominates over most of the given L/λ range, but that the body and backbone become significant at the higher L/λ's. It is apparent that Haslett's measurements of the acoustic cross-sections of sticklebacks and guppies vary widely and do not follow any of his curves. This variability is not fully explained by any of his experiments on fish or models. Obviously, the nature of the echo-formation process is quite complex if Haslett cannot explain this variability after such comprehensive work.

In attempting to quantify fish resources the objects of interest are usually fish schools rather than individual fish. When the target strength of a school is measured, the question to be answered is "What is the average size and number of fish required to produce this target strength?" The answer will depend on the average target strength of the fish in the school, and any variations among the individuals will be of minor importance. If a forward-looking sonar is used for quantification, the minimum size and number of fish required to produce a given target strength will occur at the aspect for which the target strength of an individual fish is a maximum. This aspect will be near the side aspect of the fish. Thus, average values for the maximum side-aspect target strength of an individual fish are important for quantification of fish schools with a forward-looking sonar. If a downward-looking sonar, or echo sounder, is used for quantification, average values for the dorsal-aspect target strength of an individual fish are important.

For these reasons the author has made maximum side-aspect (Love, 1969) and dorsal-aspect (Love, 1971) target strength measurements as a function of fish size, species, and frequency. The measurements were made in the laboratory, and hollow rubber spheres were used as reference targets for calibration. It was found that the magnitude of the variation in target strength for one species was of the same order as it was for all species. Therefore the data for all species were combined with all other available pertinent data and a regression line was calculated for each aspect using the method of least squares.

Figure 4 shows all the dorsal aspect data. The data were obtained using fish from 16 families in 8 different orders: Clupeiformes, Cypriniformes, Gasterosteiformes, Cyprinodontiformes, Mugiliformes, Gadiformes, Beryciformes, and Perciformes. The fish ranged in length from about 1 cm to 1 m. Some had swim bladders while others did not. Insonifying frequencies ranged from 8 kHz to 1480 kHz. Note that the parameters used here are σ/λ^2 and L/λ, which differ slightly from those used by Haslett. The equation for the regression line calculated from these data is

$$\sigma/\lambda^2 = 0.041\ (L/\lambda)^{1.94}, \qquad (9)$$

and the dorsal-aspect target strength is

$$T_D = 19.4 \log L + 0.6 \log \lambda - 24.9 \qquad (10)$$

Equation (10) is for T_D at 1 m and L and λ in meters and is valid in the range $0.7 \leqslant L/\lambda \leqslant 90$.

Figure 5 shows all the maximum side-aspect data. The data were obtained using fish from 13 families in 7 different orders: Cypriniformes, Gasterosteiformes, Cyprinodontiformes, Gadiformes, Beryciformes, Perciformes, and Pleuronectiformes. Fish size and acoustic frequency ranges were approximately the same as those for dorsal aspect. The equation for the regression line calculated from these data is

$$\sigma/\lambda^2 = 0.064\ (L/\lambda)^{2.28}, \qquad (11)$$

and the maximum side-aspect target strength is

$$T_S = 22.8 \log L - 2.8 \log \lambda - 22.9 \qquad (12)$$

Equation (12) is valid in the range $1 \leqslant L/\lambda \leqslant 130$, and again T_S is at 1 m and L and λ are in meters.

Figure 6 is a nomogram solving equations (10) and (12), given the acoustic frequency, f, in kHz, and the fish length, L, in cm.

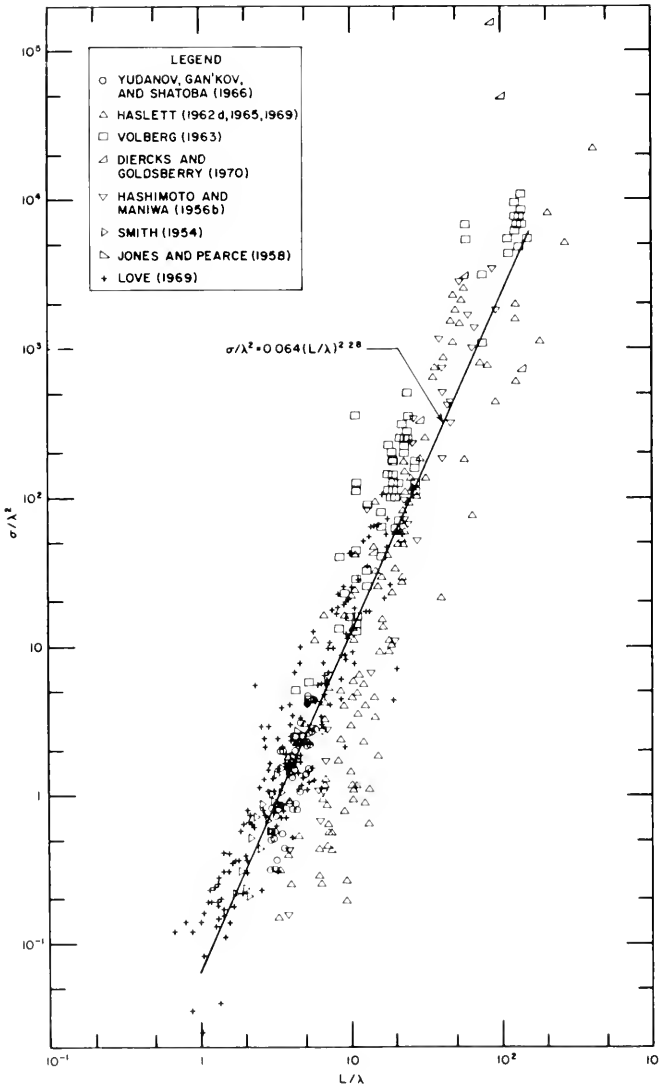

FIGURE 4.—Dorsal-aspect acoustic cross-section of an individual fish.

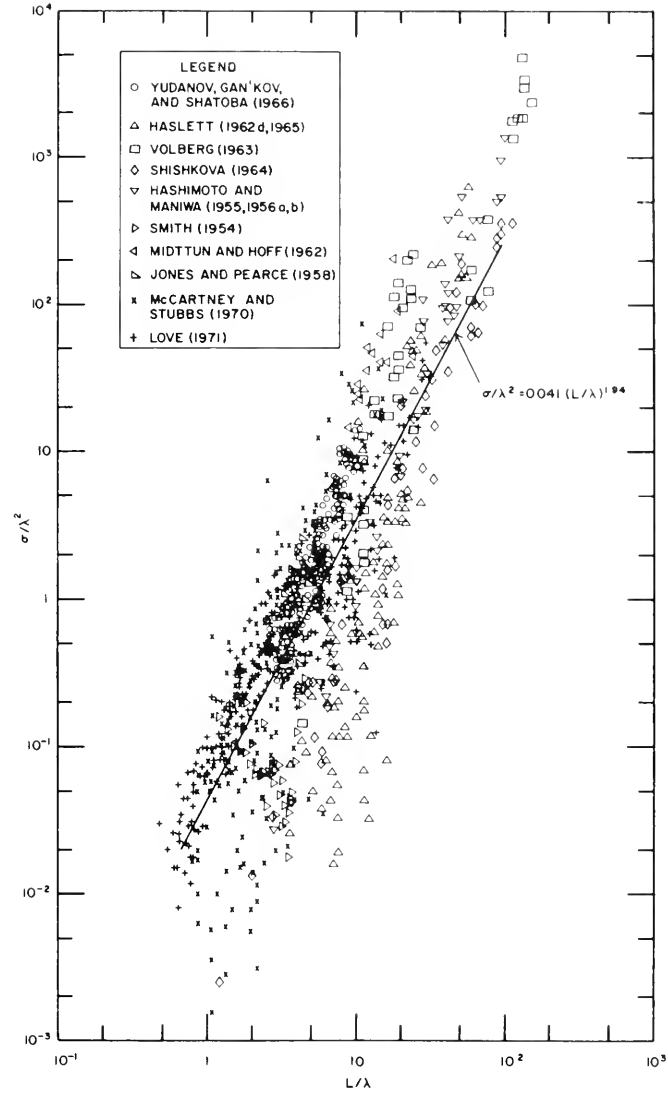

FIGURE 5.—Maximum side-aspect acoustic cross-section of an individual fish.

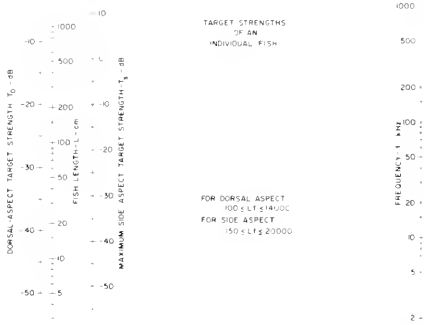

FIGURE 6.—Nomogram for calculating the dorsal-aspect and maximum side-aspect target strengths of an individual fish.

QUANTIFICATION AND IDENTIFICATION OF SCHOOLS

Since estimates for the maximum side-aspect and dorsal-aspect target strengths of individual fish are available, the determination of the target strengths of fish schools at these aspects will depend on the determination of the effects of the number of fish in the school and their distribution. If the fish are widely spaced, or in a plane perpendicular to the sonar beam, so that there is no acoustic interaction among the individuals, the target strength of the school is equal to the average target strength of an individual plus 10 times the logarithm of the number of fish. The probability of finding a school that meets these qualifications is quite small and therefore the effects of interactions among the fish must be taken into account.

Little experimental work on the acoustic interactions of fish in a school has been done, although some measurements of the target strengths of groups of fish have been made, usually with little concern for the distribution of the individuals (e.g., Thorpe and Ogata, 1967; Shishkova, 1960). Some theoretical work on distributions of scatterers has been done, the scatterers usually being point scatterers or small bubbles (e.g., Foldy, 1945; Weston, 1966). Weston (1967) has applied the results for bubbles to fish schools and has estimated reflection coefficients for regions well-below and well-above resonance. Since there is no interference region for a bubble, he does not concern himself with interference effects, and his results are of limited value in the interference region. Boyles (1969) has discussed the mathematical theory of multiple scattering from fish schools, but to obtain results in the interference region the complete spatial scattering and absorption pattern of an individual fish must be known.

The identification of a school of fish utilizing target strength information is obviously much more difficult than its quantification, and since it is not yet possible to quantify fish schools with this information, it is surely not yet possible to identify them.

Figure 3, which summarized Haslett's work, seems to indicate that no reasonable pattern of target strength vs. frequency can be found for any species due to the rapid fluctuations of target strength. Haslett's measurements were made at three widely spaced frequencies. Measurements made by the author at a larger number of more closely spaced frequencies indicate that for individual fish these fluctuations are not so rapid and that possibly individual fish may be identified through the use of target strength vs. frequency (σ L^2 vs. L λ) curves. Dorsal aspect target strength measurements made on six bay anchovies, *Anchoa mitchilli*, one Atlantic menhaden, *Brevoortia tyrannus*, five goldfish, *Carassius auratus*, and six Atlantic silversides, *Menidia menidia*, revealed that all of these fish had similar σ L^2 vs. L λ curves (Love, 1971). The most notable feature of these curves is a deep minimum in the neighborhood of L $\lambda = 10$. This minimum is easily seen in the average curves for each species shown in Figure 7. Similar measurements on three mummichogs, *Fundulus heteroclitus*, five striped killifish, *Fundulus majalis*, six black crappies, *Pomoxis nigromaculatus*, and four spotted seatrout, *Cynscion nebulosus*, revealed that the σ L^2 vs. L λ curve for any individual of these species bears no easily discernible relation to that of most, or all, of the other individuals of that species, or to the average curve for that species.

The anchovies, goldfish, and menhaden are

malacopterygians, the more primitive teleosts; the crappies and seatrout are acanthopterygians, the more advanced teleosts; and the mummichogs, killifish, and silversides belong to intermediate orders which have characteristics of both groups (Berg, 1947; Bertin and Arambourg, 1958). In general, the malacopterygians have physostomous swim bladders, osseous bone tissue, intermuscular bones, comparatively many vertebrae, fins without spines, and cycloid scales. In general, the acanthopterygians have physoclistous swim bladders, osteoid bone tissue, no intermuscular bones, comparatively few vertebrae, fins with spines, and ctenoid scales. Considering that the swim bladder, bones, and possibly scales of a fish contribute to its acoustic cross-section, it is obvious that the malacopterygians and the acanthopterygians have significant structural differences in components which have been shown to be acoustically important. Why the malacopterygians and one intermediate species display the characteristic minimum in $\sigma\ L^2$ near $L\ \lambda = 10$, or why the acanthopterygians and the other two intermediate species have no distinctive $\sigma\ L^2$ vs. $L\ \lambda$ curve cannot be answered, given the present limited knowledge of echo-formation by fish.

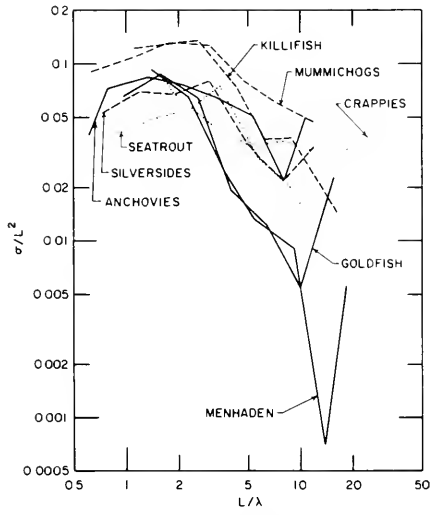

FIGURE 7.—Average measured dorsal-aspect acoustic cross-sections for different species of fish. (From Love, 1971.)

Although these differences cannot be presently explained it seems probable that if there were a geographic area in which two species with about the same size and habits predominated, and if one species were a Clupeiform and the other a Perciform, a ship with a wide-band sonar could differentiate between individuals of each species by examining their target strength vs. frequency curves. This hypothetical example indicates how very limited the present capability to identify fish by determining target strength is. Hopefully, more measurements at many frequencies, with dissection and removal of various components of the fish, and more sophisticated modeling techniques will explain the features of the target strength vs. frequency curves for individuals of a few species. This could then lead to the prediction of curves for other species, which in turn could greatly increase the ability to differentiate between individuals of different species. This information could then be applied to the differentiation of schools of different species, although it is to be expected that the manner of distribution of the fish in the school will cause significant differences between the target strength vs. frequency curve obtained for the school and the average curve for the individuals in the school.

SUMMARY

Some of the more important results of measurements of fish target strength to date are: (1) it has been determined that practically every case of interest to the fishing industry is in the region of interference effects, (2) the major contributors to the target strength of a fish in this region have been determined and their acoustic impedances measured, (3) the variations of target strength with aspect for an individual fish have been examined, (4) estimates of the dorsal-aspect and maximum side-aspect target strength of an individual fish have been made, (5) there

are indications that the identification of individual fish based on target strength vs. frequency curves is possible for limited cases.

The two goals of fish target strength measurements, namely quantification and identification of fish schools, have not yet been attained. The quantification of fish schools and the identification of many individual fish should be hopefully accomplished in the next few years. The identification of schools will require the information on quantification of schools and identification of individuals, and therefore will probably not be accomplished for some time.

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DISTRIBUTION AND BIOLOGY OF MYSIDS (CRUSTACEA, MYSIDACEA) FROM THE ATLANTIC COAST OF THE UNITED STATES IN THE NMFS WOODS HOLE COLLECTION

ROLAND L. WIGLEY AND BRUCE R. BURNS[1]

ABSTRACT

Nineteen species of marine mysids, representing 16 genera, have been assembled at the NMFS Biological Laboratory, Woods Hole, Mass. These specimens were collected between 1953 and 1969 from the continental shelf and slope off the east coast of the United States between Canada and southern Florida. The species represented are: *Eucopia grimaldii, Borcomysis tridens, Bowmaniella portoricensis, Anchialina typica, Erythrops erythrophthalma, Meterythrops robusta, Hypererythrops caribbaea, Pseudomma affine*, *Pseudomma* sp., *Amblyops abbreviata, Bathymysis renoculata, Mysidopsis bigelowi, M. furca, Promysis atlantica, Mysis mixta, M. stenolepis, Praunus flexuosus, Neomysis americana*, and *Heteromysis formosa*.

Geographic and bathymetric distributions, relations with bottom sediments, and other ecological information are given for all species. Biological data such as spawning season, clutch size, body length at maturity, and similar information are reported for 11 species. More detailed accounts of the life history and ecology of *Erythrops erythrophthalma, Mysidopsis bigelowi*, and *Neomysis americana* are made possible by the large numbers of specimens of these species.

This report is based on the collection of mysids assembled by the Food Habits Project and the Benthic Invertebrates Project at the National Marine Fisheries Service (NMFS), Biological Laboratory—formerly known as the Bureau of Commercial Fisheries (BCF)—Biological Laboratory, Woods Hole, Mass. Mysids were not specifically sought in assembling this collection; they were acquired from biological samples collected for ecological studies pertaining to various kinds of demersal fishes and assemblages of benthic invertebrates. Estuarine and inshore species are few because nearly all sampling was conducted in offshore areas.

The known mysidacean fauna off the eastern coast of the United States is not extensive. Tattersall (1951) made a thorough review of the literature and the mysid specimens in the U.S. National Museum. He reported only 11 shallowwater (less than 200 m) species occurring in the area between Maine and Florida. This includes estuarine and shore forms as well as middle and outer continental shelf species. Although a few additional species have been found in this area since the time of Tattersall's study (Klawe, 1955; Bowman, 1957, 1964; Wigley, 1963; Băcescu, 1968; Haefner, 1968; and others) and undoubtedly some species remain undetected, it is reasonable to conclude that only a modest number of different kinds of mysids occur in this region.

A substantial portion of the species in the NMFS samples from the western Atlantic also occur in European waters. They are: *Eucopia grimaldii, Borcomysis tridens, Erythrops erythrophthalma, Meterythrops robusta, Pseudomma affine, Amblyops abbreviata, Mysis mixta, Praunus flexuosus*, and *Heteromysis formosa*. Those species that do not have an amphi-Atlantic distribution are largely indigenous to the western North Atlantic, namely: *Bowmaniella portoricensis, Hypererythrops caribbaea, Pseudomma* sp., *Bathymysis renoculata, Mysidopsis bigelowi, M. furca, Mysis stenolepis*, and *Neomysis*

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americana. These indigenous species are all inhabitants of warm-temperate to tropical areas; none are subarctic or boreal. Presumably members of this group have been unable to bridge the ocean because of unfavorable water temperatures across the northern rim of the Atlantic Ocean where water depths are favorable. *Amblyops abbreviata* and *Meterythrops robusta*, in addition to having an amphi-Atlantic distribution, are also widely distributed in the North Pacific Ocean. Only *Anchialina typica*, which occurs in the Atlantic and Pacific Oceans, and *Promysis atlantica*, which occurs in the North and South Atlantic Oceans, do not fall into the two main categories above.

The European species *Praunus flexuosus*, now well established in the coastal waters of New England, may have been introduced rather recently by the activities of man (discussion on pages 735 and 736).

The information presented herein supplements our scanty knowledge of the biology of individual species and also provides a general review of the kinds of mysids in the western Atlantic, their distribution, relative abundance, and relations with some environmental characteristics.

MATERIALS AND METHODS

Three million specimens representing 19 species and 16 genera were collected during 1953-69 from the continental shelf and slope off the eastern coast of the United States between Canada and southern Florida.

Two-thirds of the samples were collected from the offshore New England region, between Nova Scotia, Canada, and Long Island, N.Y. (Figure 1). About 2,000 samples were analyzed from this region. Sparse sampling (1,000 samples) was conducted between New York and Key West, Fla. Most of the collections were taken by six oceanographic research vessels: *Albatross III*, *Albatross IV*, *Bluebuck*, and *Delaware*, all operated by the National Marine Fisheries Service; and *Asterias* and *Gosnold*, operated by the Woods Hole Oceanographic Institution, Woods Hole, Mass. Collection data and biological information for each sample of mysids in the NMFS collection are given in Burns and Wigley.[2] The collection data include: latitude and longitude, water depth, date, sampling gear, vessel name, cruise and station number. The biological information consists of the number of specimens, summary of body length by species, sex, and stage of maturity.

The 12 kinds of sampling instruments used in collecting mysids were: bottom skimmer, Campbell grab, dip net, 30-cm ring net, 1-m ring net, plankton net, naturalists dredge, otter trawl, sled-mounted ring net, Smith-McIntyre grab, shrimp trawl, and Van Veen grab. A few specimens were obtained from fish stomachs. The kinds of gear most successful in catching mysids were ring nets, grab samplers, the bottom skimmer (a combination dredge and plankton net), and dredges with fine-mesh nets. Only occasional specimens were obtained with bottom trawls and dredges with coarse mesh nets.

Mysids were preserved in Formalin at sea and transferred to ethyl alcohol at the time the samples were sorted in the laboratory ashore.

In classifying larvae according to their stage of development, we have followed Nair (1939).

A total of 5,566 specimens were examined under low-power magnification with a binocular microscope to determine sex and stage of maturity and to measure size. Body length was measured from the anterior margin of the carapace to the posterior end of the telson, using an ocular micrometer in the microscope.

SYSTEMATIC ARRANGEMENT

For the systematic arrangement and terminology we have followed Tattersall and Tattersall (1951). The list of species in their respective groupings are as follows:

[2] Burns, Bruce R., and Roland L. Wigley. 1970. Collection and biological data pertaining to mysids in the collection at the BCF Biological Laboratory, Woods Hole, Lab. Ref. No. 70-2, 36 p. Bur. Commer. Fish. Biol. Lab., Woods Hole, Mass. (Unpublished manuscript.)

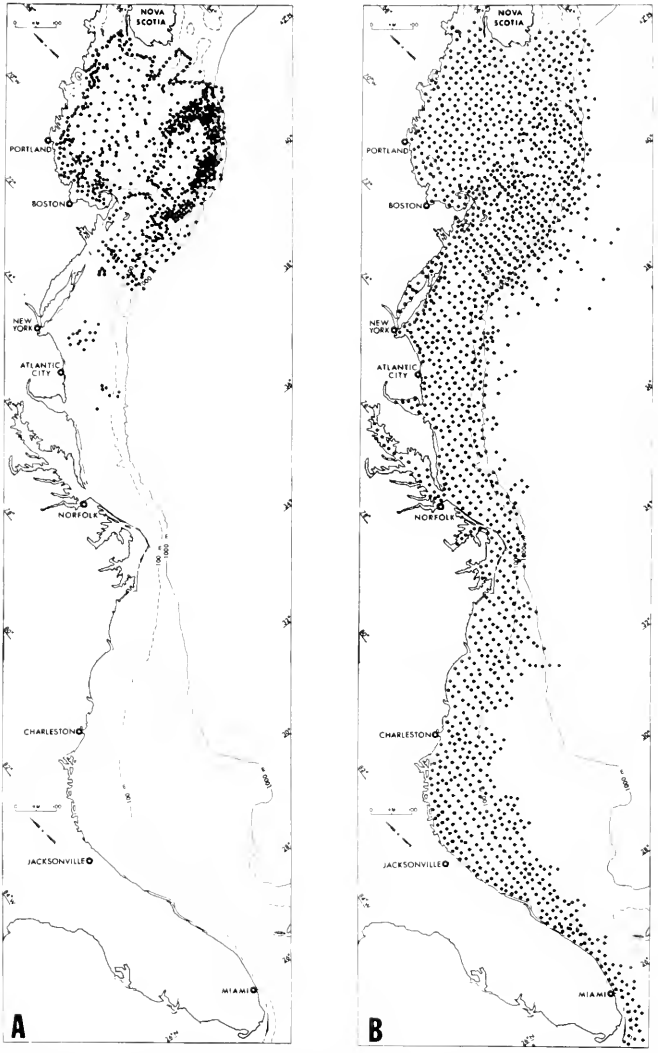


FIGURE 1.—Chart of the Atlantic Continental Shelf and adjacent area showing the location of sampling stations. A. Samples collected with dredges and similar types of collection instruments. B. Samples collected by means of grab samplers.

Order MYSIDACEA

Suborder LOPHOGASTRIDA

Family EUCOPIIDAE

Eucopia grimaldii Nouvel, 1942

Suborder MYSIDA

Family MYSIDAE

Subfamily BOREOMYSINAE

Boreomysis tridens G. O. Sars, 1870

Subfamily GASTROSACCINAE

Bowmaniella portoricensis Băcescu, 1968

Anchialina typica (Kroyer, 1861)

Subfamily MYSINAE

Tribe ERYTHROPINI

Erythrops erythrophthalma (Goës, 1861)

Meterythrops robusta S. I. Smith, 1879

Hypererythrops caribbaea Tattersall, 1937

Pseudomma affine G. O. Sars, 1870

Pseudomma sp.

Amblyops abbreviata (M. Sars, 1869)

Tribe LEPTOMYSINI

Bathymysis renoculata Tattersall, 1951

Mysidopsis bigelowi Tattersall, 1926

Mysidopsis furca Bowman, 1957

Promysis atlantica Tattersall, 1923

Tribe MYSINI

Mysis mixta Lilljeborg, 1852

Mysis stenolepis S. I. Smith, 1873

Praunus flexuosus (O. F. Müller, 1776)

Neomysis americana (S. I. Smith, 1873)

Tribe HETEROMYSINI

Heteromysis formosa S. I. Smith, 1873

SPECIES ACCOUNTS

Order MYSIDACEA

Suborder LOPHOGASTRIDA

Family EUCOPIIDAE

Eucopia grimaldii Nouvel, 1942

This mysid is a moderately large bathypelagic species that occurs most commonly at depths of about 2,000 m and has not been found at less than 300 m. Its geographic distribution is cosmopolitan, it having been reported from the North and South Atlantic, Pacific, and Indian Oceans. The majority of records are from temperate and tropical waters, but it has been recorded from as far north as Iceland and southern Greenland and as far south as South Africa and New Zealand (Fage, 1942; Tattersall, 1951).

There is only one specimen of *E. grimaldii* in our collection (Burns and Wigley, Table 2), taken at a deepwater station (700 m) along the continental slope off southern New England (Figure 2). This specimen was caught in a 12.2-m shrimp trawl equipped with a coarse-mesh (6.5 cm extension measure) net. Although the net was fished on bottom, it cannot be determined at what depth the specimen occurred. The low fishing efficiency of this trawl during setting and retrieval, however, lends support to the belief that it was caught on or near the ocean bottom.

This is only the second record of this species from off the eastern coast of the United States, even though it is moderately common in other areas and widely distributed throughout the world. Its occurrence in deep water over bottom sediments composed of silts and clays is typical for this species.

Suborder MYSIDA

Family MYSIDAE

Subfamily BOREOMYSINAE

Boreomysis tridens G. O. Sars, 1870

This is a moderately large species that is known to occur only in the North Atlantic Ocean. It is distributed along the eastern Atlantic Continental Slope from the Bay of Biscay

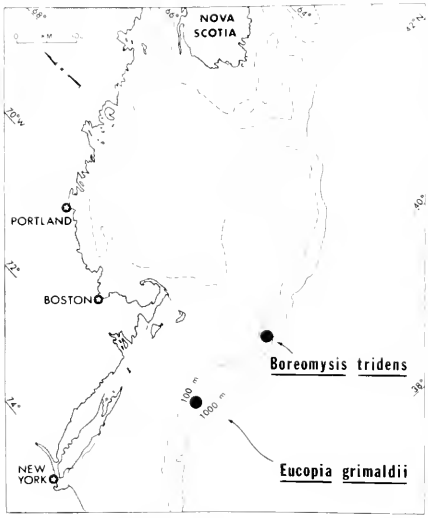


FIGURE 2.—Geographic distribution of *Eucopia grimaldii* and *Boreomysis tridens* based on specimens in the collection at the NMFS Biological Laboratory, Woods Hole.

north to Norway, across to the Faroe Islands, south and west of Iceland and Greenland, southward along the continental slope of North America (Tattersall and Tattersall, 1951). Tattersall (1951) lists over 20 records from off the northeast coast of the United States, ranging as far south as Delaware (lat 38°27′ N).

This species was reported by Verrill (1885) as being common on the continental slope in the western Atlantic. The collections of this mysid reported by Tattersall (1951), all made by the research vessels *Albatross*, *Fish Hawk*, and *Challenger*, reflect its deepwater habitat.

Our collection contains one sample of this species consisting of three specimens (Burns and Wigley, Table 3). They were taken on the west side of Hydrographer Canyon, located about 130 km southeast of Nantucket, Mass. (Figure 2). The depth of water at this location is 402 m. Its occurrence at this depth and on bottom sediments of silty sand are characteristic for this species. Two specimens 15.0 and 16.5 mm in length are immature; a 26-mm specimen is an adult male.

Subfamily GASTROSACCINAE

Bowmaniella portoricensis Băcescu, 1968

B. portoricensis is morphologically very similar to *B. johnsoni* (Tattersall). A close examination of pleopod 3 in male specimens and of the uropod and the posterior part of the carapace in all specimens was required for reliable differentiation of these two species. *B. portoricensis* is a subtropical species that has been reported by Băcescu (1968) off the southeastern coast of the United States between Beaufort, N.C., and northern Florida. Although this species was known in only a few locations at the time of Băcescu's report, it is undoubtedly more common than these few published records suggest, as indicated by the relatively large number of samples in the NMFS collection. Its rather small size (up to 11-mm body length) and its occurrence in areas that have not yet been thoroughly studied presumably have contributed to the paucity of collections.

The NMFS collection contains 100 specimens of *B. portoricensis* from 16 samples, all of which are from off the southeastern coast of the United States (Figure 3; Burns and Wigley, Table 4). The northernmost samples were taken approximately 90 km north of Cape Hatteras, N.C., at lat 36° N. The southern limit of our samples is 15 km south of Ft. Pierce, Fla., at lat 27°20′ N. All of these samples were collected within 125 km of the shoreline. Water depths at which they occurred range from 9 to 56 m, and most of them were taken at depths between 15 and 39 m.

Body lengths average 6.9 mm; the range in length is 3.1 to 10.0 mm.

This species is chiefly an inhabitant of sandy sediments. Sand was present at all stations. Shell was a major component at 7% of the stations and a minor component at 26% of the stations where *B. portoricensis* occurred.

Seven females, 7.9 to 10 mm in length, from the May and June samples were carrying larvae in the brood pouch. The number of larvae per clutch ranges from 1 (obviously an incomplete brood) to 30. Only the relatively advanced

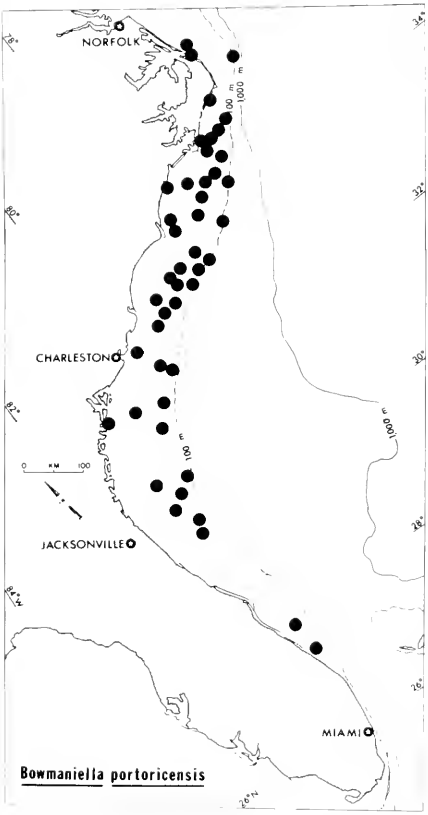


FIGURE 3.—Geographic distribution of *Bowmaniella portoricensis* based on specimens in the collection at the NMFS Biological Laboratory, Woods Hole.

larval stages—V, VI, and VII, with lengths from 1.0 to 1.2 mm—are represented. The presence of larvigerous females as well as immature specimens in May and June samples reveals that *B. portoricensis* spawns not only in early summer, but in the springtime as well.

The NMFS collection contains 31 males and 17 females, a ratio of 0.7 male to 1 female.

Anchialina typica (Krøyer, 1861)

This moderately small, stout mysid is widely distributed in both the Atlantic and Pacific Oceans. It has been reported from the Pacific near the central (Hawaii and Gilbert Islands) and southwestern (China Sea to Great Barrier Reef) regions. According to Tattersall (1951) it is abundant in the region of the Philippine Islands and the East Indies. Though it has been reported in the North Atlantic from south of Newfoundland (Nouvel, 1943), records from the vicinity of the Bahama Islands and Cuba are the most common. Many occurrence records in the literature are based on specimens collected in surface waters.

The NMFS collection contains three specimens from three different stations (Figure 4;

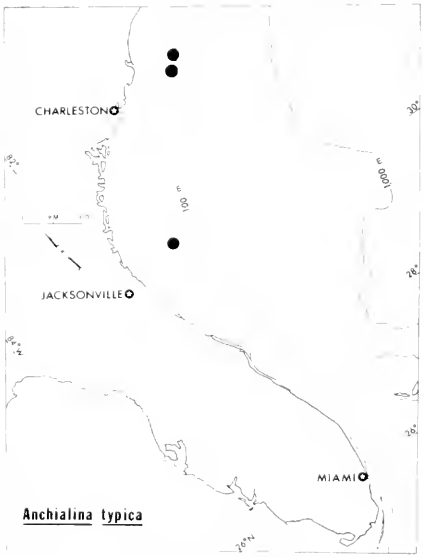


FIGURE 4.—Geographic distribution of *Anchialina typica* based on specimens in the collection at the NMFS Biological Laboratory, Woods Hole.

Burns and Wigley, Table 5). The southernmost sample is from off northern Florida. The other two samples were taken 60 to 70 km east of Georgetown, S.C. All specimens are males 4.5 to 5.0 mm long. They were all collected with the Campbell grab in rather shallow water, between 32 and 38 m. Bottom sediments at these stations are composed of fine and medium sand. These are the first records of this genus and this species from the shallow shelf region off the eastern coast of the United States.

A closely related species, *A. agilis*, is an active swimmer that migrates to surface waters at night and descends to deep water before daybreak (Russell, 1925). Based on NMFS records of *A. typica* reported herein, and on Tattersall's (1951) records, it appears likely that *A. typica* in the shallow and moderately shallow regions of the continental shelf may also dwell on bottom during the day and rise to surface or near surface waters at night.

Subfamily MYSINAE

Tribe ERYTHROPINI

Erythrops erythrophthalma (Göes, 1864)

Geographic Distribution

This colorful mysid species has a widespread distribution on the continental shelf and upper portion of the continental slope in Arctic seas and the North Atlantic Ocean. In eastern Atlantic waters it extends from the Arctic southward to the British Isles. In the western Atlantic it has been reported from off Greenland, eastern Canada, and off the northeastern United States as far south as Delaware (Gardiner, 1934; Bigelow and Sears, 1939; Tattersall, 1951; Tattersall and Tattersall, 1951).

The NMFS collection contains 187 samples totaling 1,573 specimens of this species (Figure 5; Burns and Wigley, Table 6). These samples were collected on the continental shelf and slope between southeastern Nova Scotia and Long Island, N.Y. By far the largest number of samples is from the southern part of Georges Bank. A moderate number of samples were taken in the offshore southern New England area south

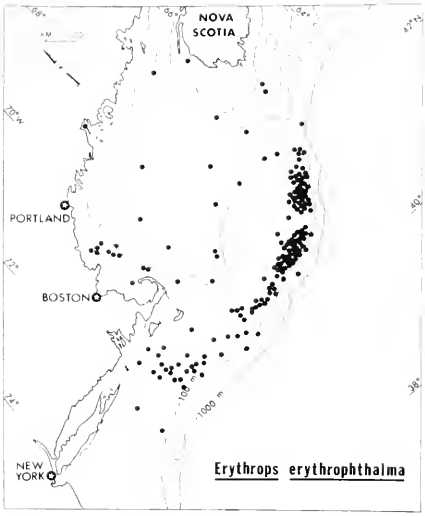


FIGURE 5.—Geographic distribution of *Erythrops erythrophthalma* based on specimens in the collection at the NMFS Biological Laboratory, Woods Hole.

of Rhode Island and eastern Massachusetts. The common occurrence of this species on Georges Bank was somewhat unexpected in view of the fact that Whiteley (1948) found it to be a minor component in plankton samples collected there in 1939-41.

Bathymetric Distribution

This is an offshore species that occurs chiefly in mid- to outer-shelf depths. The shallowest record in published accounts that we have seen is 12 m, reported by Procter (1933) for specimens collected on the coast of Maine. Deepest record previously reported is 275 m, from the Gulf of St. Lawrence (Tattersall, 1957). Depth range for samples in the NMFS collection is 18 to 421 m (Table 1).

A large proportion (78% of the samples and 90% of the specimens) were taken at midshelf depths, 60 to 100 m. The sample from unusually

TABLE 1.—Bathymetric distribution of *Erythrops erythrophthalma*, based on the NMFS collection.

Water depth	Samples	Specimens
m	*Number*	*Number*
0- 19	1	1
20- 39	1	2
40- 59	13	277
60- 79	73	1,676
80- 99	72	2,422
100-119	12	133
120-139	4	6
140-159	4	33
160-179	3	17
180-199	1	1
200-219	1	2
220-239	1	1
420-439	1	2
Total	187	4,573

deep water was collected at the head of Lydonia Canyon, along the southwestern edge of Georges Bank. This sample consisted of two specimens, one adult female and an immature, collected in a stramin-mesh ring net towed 1 to 2 m above the sea bottom.

Spawning

Seven ovigerous specimens are present only in the August samples, and 11 larvigerous specimens are present in August and September samples. This is an unusually small number of specimens (about 1%) in spawning condition compared with the total number of adult females. Furthermore, the spawning females are not especially large. Their average length is 6.4 mm, range 5.7 to 7.1 mm. Larger specimens taken during the same month and at other seasons are not in spawning condition. The presence of immature specimens of about 4 to 5 mm in length throughout the period from June through December implies an extended spawning season during the warmer part of the year, plus the possibility of spawning during other seasons as well. The absence of well-defined length modes from month to month also supports the hypothesis of a lengthy spawning period.

The number of eggs per clutch ranges from 1 to 6, and the number of larvae from 2 to 15. The lower values appear to represent incomplete broods that resulted from losses incurred during the catching and processing procedures.

In summary, the available evidence from the NMFS material suggests that only a small part of the population spawns at one time and that spawning takes place throughout a large part of the year—possibly from spring through fall or longer.

Sex Ratio

The NMFS collection contains 1,536 males and 1,692 females, a ratio of 0.91 male to 1 female.

Body Size

The eggs are nearly spherical and average 0.4 mm in diameter. Larvae of stage V are 1.2 mm long, and stage VII are 1.4 mm. Immatures range in length from 3.0 to 6.1 mm. Mature specimens have an overall size range from 4.3 to 9.6 mm. Size range during various seasons, separated by sexes, are:

Season	Males	Females
Mar.-Apr.	6.4	6.1
May-June	5.2-7.5	5.8-7.0
July-Aug.	5.1-8.3	5.0-9.0
Sept.-Oct.	4.8-9.6	4.3-8.7
Nov.-Dec.	5.1-8.9	5.0-8.9

A size comparison of mature males with mature females from the same samples discloses that males are 0.3 to 0.4 mm larger than females.

The minimum size of adults is generally larger in the early summer, decreases in late summer and fall, then increases again in early winter. This is due to an earlier maturation of immature individuals during the period when water temperatures are comparatively high. The trend for maximum size of adults is just the opposite. Maximum length is smallest in early summer, increases in the late summer and fall, and (the males only) decreases again in early winter. It is not clear whether this sequence in size differences results solely from faster growth during the warm season, or whether a summer generation has reached the culmination of its life span in September-October.

Length of Life

An analysis of body length measurements did not disclose trends in growth or separate length-frequency modes that indicated year classes. Immature specimens with an average length of roughly 4.5 mm are present in June, August, September, November, and December. The mature groups of both males and females in these same months are mostly between 5 and 7 mm long and do not exhibit the expected increases in size as the seasons progress. Our tentative conclusion is that *E. erythrophthalma* has a short life span, a rapid growth rate, and a lengthy spawning season.

Relation to Bottom Sediments

Members of this genus are considered to dwell on or near the sea bottom, and our catch records substantiate this view. It is uncommon in plankton samples collected in the upper water layers, but is regularly taken in hauls collected near bottom. It occurred most frequently and in greatest abundance on sand sediments (Table 2). Seventy-seven percent of the samples and over 90% of the specimens were taken on, or over, sand sediments. A moderate number of samples (10%) were taken in areas of fine-grained sediments. Furthermore, it should be added that most of the sediments along southern Georges Bank, where the majority of samples were taken, contain modest amounts of silt, generally between 1 and 10%. These quantities are insufficient to be incorporated in the bottom type terminology (Shepard, 1954). Although these are small quantitities, the presence of silt on the sediment surface makes it readily available to the mysids. Furthermore, this species is common on the silty sediments in the region south of Martha's Vineyard, Mass. Thus *E. erythrophthalma* appears to inhabit sediments containing a small to moderate amount of silt, in contrast to *N. americana* and *M. bigelowi*, which are more common in sediments having a very low silt content.

TABLE 2.—Frequency of occurrence of *Erythrops erythrophthalma* in various types of bottom sediments, based on the NMFS collection.

Bottom type	Samples	Specimens
	Number	*Number*
Rock-gravel	6	122
Gravel-sand	6	13
Glacial till	2	12
Sand	144	4,224
Sand-silt	12	50
Silt-clay	17	152
Total	187	4,573

Meterythrops robusta S. I. Smith, 1879

This rather large but uncommon species has a wide distribution in boreal and subarctic waters. In the Atlantic region it has been reported from the Kara Sea, Spitsbergen, Norway, Greenland, Gulf of St. Lawrence, and in the Gulf of Maine as far south as Cape Cod, Mass. In the Pacific it occurs in the area between Alaska and Washington. Moreover, assuming that *M. microphthalma* is a synonym for *M. robusta* (see Banner, 1951), then its distribution also includes the region off the east coasts of Japan and Korea (Tattersall, 1951).

The NMFS collection contains nine specimens from six stations, all from the periphery of the Gulf of Maine (Figure 6; Burns and Wigley, Table 7). Four specimens were collected at one station in the channel north of Browns Bank; three specimens were from three stations north of Great South Channel; and two specimens were taken off eastern Massachusetts.

Bathymetric distribution ranged from 64 to 150 m, and all specimens except one were from depths between 110 and 150 m.

The type of bottom sediments inhabited by this species is distinctive. The bottom sediments contained gravel at five of the six stations where *M. robusta* occurred. Three of the samples were taken on glacial till; one sample was from sandy gravel; one sample was from gravel; and one sample was from silt-clay. All other offshore species of mysids in the NMFS collection are associated with sand or finer grained sediments.

All specimens of *M. robusta* in the NMFS collection were caught in stramin nets or natu-

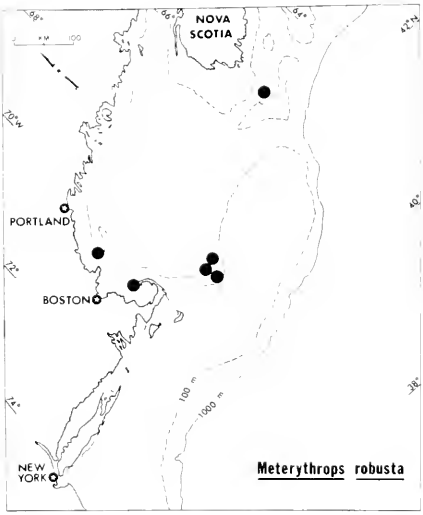


FIGURE 6.—Geographic distribution of *Meterythrops robusta* based on specimens in the collection at the NMFS Biological Laboratory, Woods Hole.

ralists dredges towed along the sea bottom. From this we infer that this species probably inhabits the sea bottom or the water stratum closely adjacent to bottom.

Body length of immature specimens ranged from 6.6 to 7.5 mm, young males 8.5 to 12.0 mm, and young females 8.6 to 9.3 mm. There were no large specimens in the NMFS collection that compare in size with the 28.5-mm adult male reported by Smith (1879) from the Gulf of Maine.

The six adult specimens included four males and two females, a ratio of two males to one female.

No definite information pertaining to spawning is available from the material in our collection. Both females are young and without external eggs. The only evidence on this subject is the presence of immature and young specimens in the August collections, which implies a spring spawning season.

Hypererythrops caribbaea Tattersall, 1937

This species is distributed along the east coast of North America from Maine to the Caribbean Sea. It has been reported from the outer continental shelf and upper slope at depths between 211 to 402 m (Tattersall, 1951). Apparently it is an uncommon species, since it has been reported previously from only seven stations. Body size is moderately small; lengths range from about 8 to 13 mm.

The NMFS collection contains three specimens from three locations (Figure 7; Burns and Wigley, Table 8), between the southern Gulf of Maine and the continental shelf margin south of Rhode Island. This southernmost sample is from a depth of 179 m; the others are from 168 to 179 m. The smallest specimen, 5.5 mm body length, is immature; the other two, 9.5 to 11.0 mm, are adult females. The largest specimen is larvigerous with an incomplete brood

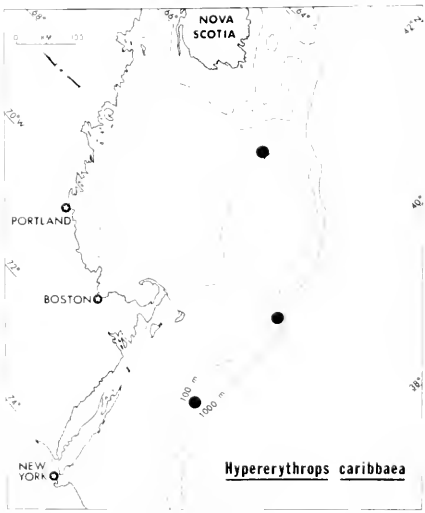
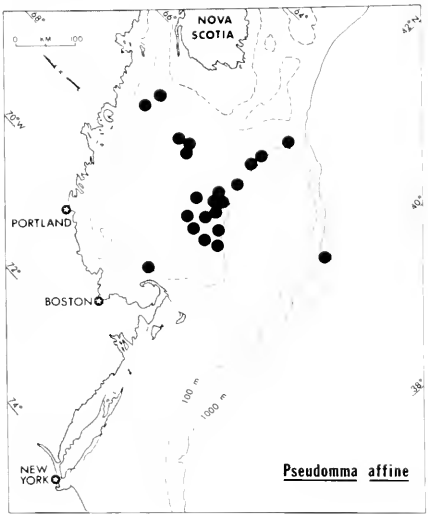


FIGURE 7.—Geographic distribution of *Hypererythrops caribbaea* based on specimens in the collection at the NMFS Biological Laboratory, Woods Hole.

of only two young in the brood pouch. Collection of this specimen in August indicates a summer spawning season for this species. This larvigerous specimen was found in the stomach of a Gulf Stream flounder, *Citharichthys arctifrons* Goode. Since *H. caribbaea* is found in warm-temperate waters, its occurrence in the Gulf of Maine may be restricted to the deeper basin areas where water temperatures are ameliorated by the intrusion of relatively warm, high-salinity slope water.

Pseudomma affine G. O. Sars, 1870

The geographic distribution of this species extends from the Bay of Biscay northward to Norway, westward to Iceland, Greenland, and North America. We found only two records of its occurrence in North American waters. Tattersall (1951) recorded one specimen taken by the research vessel *Fish Hawk* at station 999, located 153 km south of Rhode Island (lat 39°45′ N, long 71°30′ W) at a depth of 487 m. Klawe (1955) reported taking this species 2 km off Campobello Island, New Brunswick, Canada.



Bathymetric range of all records from the North Atlantic Ocean is 80 to 914 m.

Our collection contains 119 specimens from 22 samples (Figure 8; Burns and Wigley, Table 9), all from the Gulf of Maine, except one from just south of Georges Bank. They were collected at moderate water depths: 146 to 329 m. Bottom sediments at these collecting sites contain large proportions of fine particles, chiefly silts, clays, and fine sands. Only two samples were taken in areas having gravel or coarse sand bottom.

Body lengths range from 4.0 to 13.1 mm; the majority are between 8 and 11 mm.

Evidence of an extended spawning season is provided by the presence of ovigerous, larvigerous, and small juvenile specimens in the collection. A 10.9-mm ovigerous specimen was taken in December (*Albatross III*, cruise 70, station 41) with 11 eggs and an average egg diameter of 0.4 mm. One 11.0-mm larvigerous specimen taken in August (*Albatross IV*, cruise 65-11, station 56) contains one larva 1.2 mm long. Additionally, juveniles 5 mm or less in length were taken in August, November, and December. Spawning thus takes place during summer and winter and may also occur in the spring and fall.

Sex ratio of the specimens in the NMFS collection is 2.3 males (68 specimens) to 1 female (29 specimens).

Several morphological features in our specimens differ slightly from published descriptions of this species. In Table 3 are listed the number of spines on the telson and relative length of the antennal scale apex and relative width of the antennal scale. Specimens in our collection have fewer apical spines (nearly always 6) on the telson, and a greater number of spines (9-24, average 17) on the lateral margins of the telson, than specimens from the eastern Atlantic. Also, the antennal scales on Gulf of Maine specimens have a proportionately longer apex (that portion of the scale between the spine cleft and the anterior end) than eastern Atlantic specimens. Spination of the telson and shape of the antennal

FIGURE 8.—Geographic distribution of *Pseudomma affine* based on specimens in the collection at the NMFS Biological Laboratory, Woods Hole.

TABLE 3.—Number of spines on the telson and the proportional measurements of the antennal scale for individual male and female specimens of *Pseudomma affine* of various sizes.

Body length	Spines on telson		Antennal scale	
	Apex	Lateral[1]	Relative length of apex[2]	Relative width[2]
mm	*Number*	*Number*	%	%
Males				
8.0	6	15	31	29
10.0	6	17	34	25
10.3	6	19	36	27
13.0	8	24	37	29
Females				
4.3	6	9	28	32
7.0	6	13	33	33
7.3	6	--	36	31
10.4	6	21	40	29
10.5	6	19	44	30
12.0	6	18	42	30

[1] Total count of lateral spines from both sides of the telson.
[2] Measurements expressed as percentages of the antennal scale length.

scale change as the specimens increase in size. The number of lateral spines on the telson increases with body length, particularly in males. In both males and females the apex of the antennal scale is proportionately longer in larger specimens.

Sexual dimorphism is rather slight in the characters listed in Table 3. Females have a relatively larger antennal scale apex and a slightly broader antennal scale than males.

The close affinity of *P. affine* with *P. roseum* G. O. Sars necessitated considerable effort to establish the identity of the specimens at hand. In addition to the taxonomic characters mentioned above, specimens in our collection were distinguished from *P. roseum* by: the relatively broad telson and an ocular plate that has a broad, gently rounded anterolateral "corner" with serrations extending along a large portion of the lateral margins. The practice of using morphological characters that change with size or vary according to sex or stage of maturity has led to confusion between these species. Further taxonomic studies of these two species are clearly in order.

Pseudomma sp.

Ten specimens of *Pseudomma* that do not correspond morphologically to any known species are represented from three localities off southern New England. A description of this species and notes on its ecology is being prepared and will be reported elsewhere.

Amblyops abbreviata (M. Sars 1869)

This widely distributed boreal mysid occurs in both the North Atlantic and North Pacific Oceans. In the Pacific it has been found off Japan and off the west coast of North America from Washington to Alaska. In the Atlantic it ranges from the Bay of Biscay north to Scandinavia, west to Greenland and North America. Previous records from the northeastern coast of the United States consist of 38 specimens from 7 offshore stations situated between Cape Cod, Mass., and northern New Jersey. These specimens were collected by the U.S. Fish Commission between 1879 and 1881 at water depths between 238 and 838 m.

Our collection contains 34 specimens from 8 samples taken in the Gulf of Maine, primarily in the southern part of the Gulf (Figure 9; Burns and Wigley, Table 10). Water depths at these localities range from 183 to 329 m. The bottom sediments where these specimens were taken are composed predominantly of silts, clays, and fine sands; one exception is a sandy gravel bottom off the eastern end of Georges Bank where one juvenile specimen was obtained. Body length ranged from 4.7 to 15.0 mm. The size of adults is 10 to 15 mm; the males tend to be slightly larger (average length 12.9 mm) than females (average length 12.3 mm).

Spawning occurs during winter and possibly in other seasons as well. One ovigerous female collected in December (*Albatross III*, cruise 70, station 25) had 29 eggs in the marsupium, each 0.4 mm in diameter. The presence of juvenile specimens 4 to 6 mm in length in August and an 8-mm specimen in December suggests that spawning also takes place in summer and fall.

Sex ratio of the specimens in the NMFS collection is 0.65 male (11 specimens) to 1 female (17 specimens).

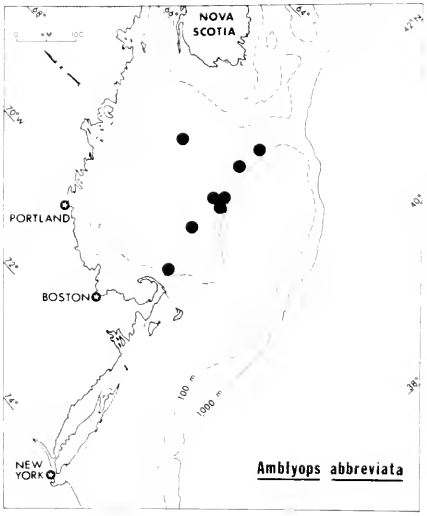


FIGURE 9.—Geographic distribution of *Amblyops abbreviata* based on specimens in the collection at the NMFS Biological Laboratory, Woods Hole.

between Hudson Canyon and Hydrographer Canyon—in the same area where they were found to be most common by the *Fish Hawk* and *Albatross* (Figure 10; Burns and Wigley, Table 11). The depth range of their occurrence is 220 to 366 m. Because two samples were collected with the bottom skimmer, it is certain these specimens were present on the sea bottom at the time of capture. Their presence on the bottom during hours of low light level, 1600 to 2200 hr, suggests they may not undertake a diurnal vertical migration. Five specimens are adults, 13.0 to 16.2 mm long, and three specimens are immature, 4.0 to 6.0 mm long. We have no information on spawning other than the presence of 4- to 6-mm specimens in June, from which a spring spawning may be inferred. Bottom sediments at the collecting sites are fine-grained types: sand, silt, and silt-clay.

Tribe LEPTOMYSINI

Bathymysis renoculata Tattersall, 1951

This large-eyed species of *Bathymysis*, which occurs only in the western Atlantic Ocean, has been reported from southern New England to the southern tip of Florida (Tattersall, 1951). The principal area of occurrence is along the outer continental shelf and upper slope between Hudson Canyon and Hydrographer Canyon (southeast of Nantucket, Mass.). The bathymetric range reported for this species is 220 to 483 m. These records are based on collections made by the research vessels *Fish Hawk* and *Albatross* during the latter part of the last century.

The NMFS collection contains eight specimens from three stations off southern New England

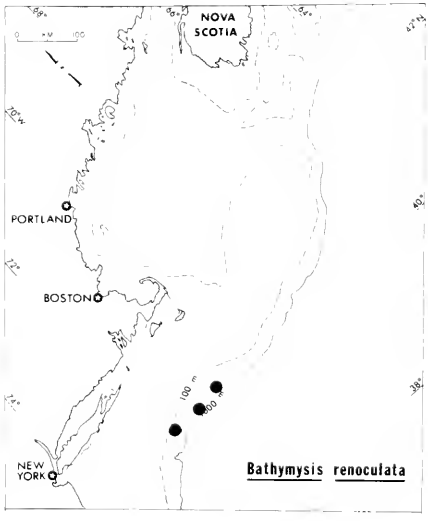


FIGURE 10.—Geographic distribution of *Bathymysis renoculata* based on specimens in the collection at the NMFS Biological Laboratory, Woods Hole.

Mysidopsis bigelowi Tattersall, 1926

Geographic Distribution

M. bigelowi is a small American species that occurs along the Atlantic and Gulf coasts of eastern and southern United States from New England to Louisiana.

The NMFS collection contains 2,031 specimens out of 54 samples (Figure 11; Burns and Wigley, Table 12) collected from northeastern Georges Bank southward along the coast to the vicinity of Jacksonville, Fla. Georges Bank is the principal area represented in the sampling; the results extend the known range of this species a considerable distance to the northeast. Only five samples were from localities south of the Nantucket Shoals region: these were taken off New Jersey, Virginia, North Carolina, and northern Florida.

It is interesting to note this species is not listed as present in the plankton samples collected from Georges Bank between 1939 and 1941 (Whiteley, 1948). Although the absence could have been due to annual fluctuations in abundance in this northern sector of its range, our results show the species was present each year we conducted moderate or heavy sampling: in 1955 through 1958, and in 1964 through 1967. More likely the absence in the 1939-41 samples resulted not from temporary fluctuations but because these earlier collections were primarily from middle and upper water levels, whereas most of the NMFS collections were taken on or near the sea bottom.

Bathymetric Distribution

M. bigelowi is a shallow-shelf species that has been reported from inshore localities such as the inlet to Indian River Bay, Del., and Calcasieu Pass, La., and from offshore waters as deep as

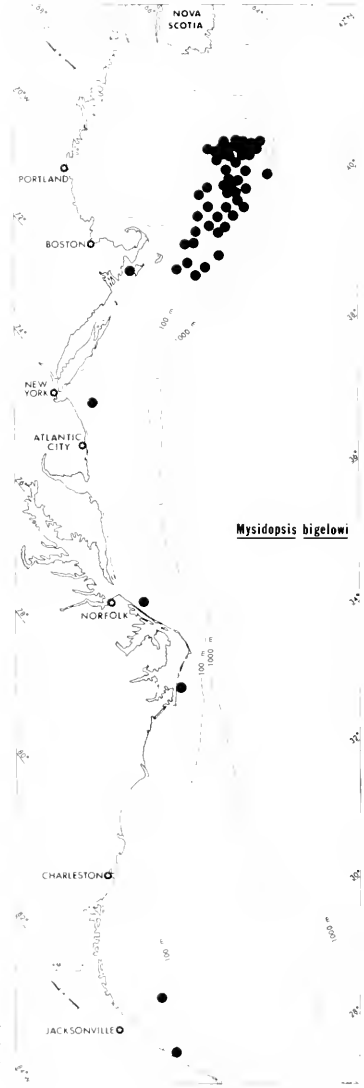


FIGURE 11.—Geographic distribution of *Mysidopsis bigelowi* based on specimens in the collection at the NMFS Biological Laboratory, Woods Hole.

77 m (Tattersall, 1951; Grice and Hart[3]; Hopkins, 1965).

Depth range of the NMFS samples is from 13 to 179 m. By far the largest number of samples (73%), greatest number of specimens (91%), and highest densities occur between 30 and 80 m (Table 4). One collection taken at an unusually deep locality, 179 m, is from the northern edge of Georges Bank. The water circulation in this area and its proximity to shallow waters commonly inhabited by this species may account for its presence at that relatively great depth.

TABLE 4.—Bathymetric distribution of *Mysidopsis bigelowi*, based on the NMFS collection.

Water depth	Samples	Specimens
m	*Number*	*Number*
10- 19	4	28
20- 29	3	10
30- 39	9	557
40- 49	8	493
50- 59	9	640
60- 69	7	73
70- 79	6	85
80- 89	4	22
90- 99	2	116
100-109	1	1
170-179	1	6
Total	54	2,031

Spawning

This species spawns from April to November in the coastal areas of Delaware (Hopkins, 1965). There is no information about spawning habits of the offshore and northern populations, and we did not find a single ovigerous or larvigerous specimen in the NMFS collection. We do, however, have immature specimens represented in samples collected in May, August, September, November, and December. This is indicative of a long spawning season, probably from early summer through fall.

The large size of immatures, 4.0 to 5.0 mm, in May samples is believed to represent the overwintering young. The absence of ovigerous females in the many large samples collected in December is evidence that spawning most likely has terminated by that time of year.

Sex Ratio

The NMFS collection contains 313 males and 195 females, a ratio of 1.6 males to 1 female.

Body Size

This species is the smallest mysid in the NMFS collection. A summary of body length data by month of capture for males, females, and immatures is given in Table 5. Range in body length among all specimens in the collection is 2.4 to 6.6 mm. The average length of immatures is 3.8 mm, males 4.9 mm, and females 5.1 mm. Members of this species were found to mature at 3.5 mm, smaller than any other species in the NMFS collection.

TABLE 5.—Means and ranges of body length of *Mysidopsis bigelowi* by months and sexes.

Month	Body length					
	Immatures		Males		Females	
	Mean	Range	Mean	Range	Mean	Range
	mm	*mm*	*mm*	*mm*	*mm*	*mm*
May	4.6	4.0-5.0	5.1	4.9-5.7	5.5	5.4-5.7
Aug.	4.0	--	4.9	4.2-6.6	4.5	3.8-5.8
Sept.	3.0	--	4.6	--	5.0	--
Nov.	3.8	2.4-4.1	5.2	4.6-5.7	5.1	4.0-6.3
Dec.	3.5	2.4-4.5	4.8	3.5-5.8	4.5	3.5-5.6

Length of Life

Our samples are inadequate to give an accurate estimate of the length of the life cycle. The few available clues, such as the presumably long spawning season and the large size of the overwintering immatures, are suggestive of a life cycle similar to *Neomysis americana*: a short-lived summer generation and a long-lived winter generation.

Relation to Bottom Sediments

Many specimens in the NMFS collection were taken with bottom samplers (Smith-McIntyre, Van Veen, and Campbell grabs) or gear that

[3] Grice, George D., and Arch D. Hart. 1962. The abundance, seasonal occurrence and distribution of the epizooplankton between New York and Bermuda. Appendix to Ref. 62-4, Woods Hole Oceanogr. Inst. (Unpublished manuscript.)

sampled water layers adjacent to the sea bed (sled net). These results suggest *M. bigelowi* lives in or on the bottom sediments during much of its life.

An analysis of the types of bottom sediments at the collecting sites reveals a high incidence of this mysid on various grades of sand (Table 6), usually sand containing little or no silt or clay. They tend to avoid fine-grained sediments as further evidenced by their absence in several hundred samples taken from a 1,000 square km area of predominantly silt and sandy-silt sediments south of Martha's Vineyard, Mass., on research vessel *Delaware* cruise 62-7.

TABLE 6.—Frequency of occurrence of *Mysidopsis bigelowi* in various types of bottom sediments, based on the NMFS collection.

Bottom type	Samples	Specimens
	Number	*Number*
Rock-gravel	4	177
Gravel-sand	1	1
Shell-sand	1	1
Sand	44	1,847
Sand-silt	1	1
Unclassified	3	4
Total	54	2,031

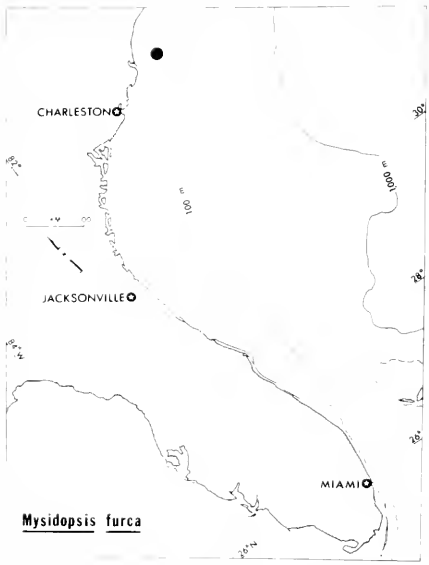


FIGURE 12.—Geographic distribution of *Mysidopsis furca* based on a specimen in the collection at the NMFS Biological Laboratory, Woods Hole.

Relation to Water Temperature

M. bigelowi inhabits water temperatures from about 2° C in the northern part of its range to summer water temperatures of about 30° C in the Florida and Louisiana areas. The annual change in temperature is slightly less than 20° C in the north and slightly more than 20° C in the south.

Mysidopsis furca Bowman, 1957

This species was described by Bowman (1957) from a sample containing 23 specimens collected in 1953 by the research vessel *Theodore N. Gill*. The specimens were obtained at one station (number 57) located 40 km from shore off the northern coast of South Carolina. Depth of water at the collecting site is 22 m. It was later reported by Brattegard (1969) from off southeastern Florida at depths of 1 to 48 m.

The NMFS collection contains one specimen, a female 1.2 mm long, taken with the Campbell grab 50 km east of Georgetown, S.C., (Figure 12; Burns and Wigley, Table 13) about 50 km southwest of the type locality. The specimen was taken at a depth of 22 m on sediment composed of fine sand. This small species has a reported size range of 4.6 to 6.1 mm (Bowman, 1957). Neither the size nor the developmental stage of the NMFS specimen provides any indication of spawning season or size at maturity.

Promysis atlantica Tattersall, 1923

This rare species was described from an immature female specimen collected off Rio de Janeiro, Brazil, in 1910 (Tattersall, 1923). It was not reported again until Clarke (1956) described the male and adult female, and gave new records of occurrence for specimens col-

lected off the coasts of Louisiana, South Carolina, and North Carolina.

The NMFS collection contains three specimens from three stations located off the southeastern coast of the United States (Figure 13; Burns and Wigley, Table 14). Their geographic distribution is from just north of Cape Hatteras, N.C., to Fort Pierce, Fla. Size range is 4.5 to 5.0 mm; all are females. They were taken in shallow water, 8 to 26 m, on sandy sediments. Little is known about the biology of this species, and the few specimens in the NMFS collection provide no additional information on spawning or length of life.

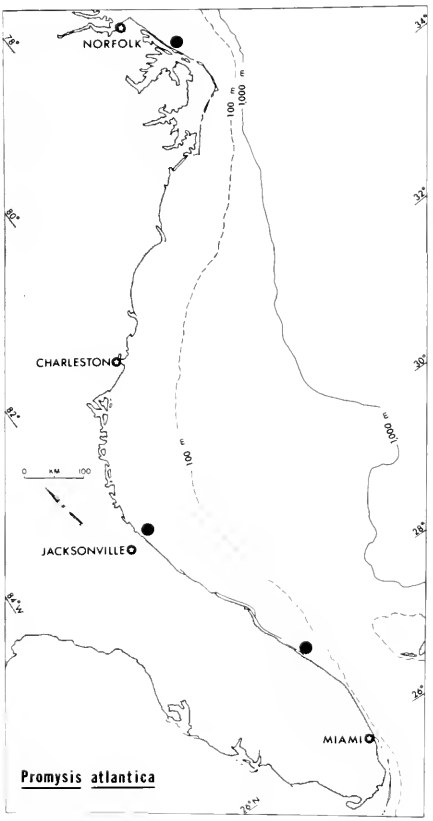


FIGURE 13.—Geographic distribution of *Promysis atlantica* based on specimens in the collection at the NMFS Biological Laboratory, Woods Hole.

Tribe MYSINI

Mysis mixta Lilljeborg, 1852

This boreal mysid occurs in the Eastern Atlantic region from the White Sea, Spitsbergen, Scandinavia and southward to the Baltic Sea, and westward to Iceland. In the Western Atlantic region it has been reported from Greenland, eastern Canada, and the eastern coast of the United States as far south as Cape Cod, Mass. In the Gulf of Maine it has been most frequently reported from off the eastern coast of Massachusetts and from a few localities off the Maine coast (Smith, 1879; Rathbun, 1905; Tattersall, 1951; and others).

This species is represented by 382 specimens from 45 samples in the NMFS collection (Figure 14; Burns and Wigley, Table 15). The majority of specimens, including all adults, were taken in the western part of the Gulf of Maine between Cape Cod, Mass., and the central Maine coast. The first records of this species from south of Cape Cod were collected in the region off Rhode Island and southeastern Long Island, N.Y. The location of the southernmost sample is lat 40°36′ N and long 71°33′ W, approximately 55 km southeast of Montauk Point, N.Y. All 26 specimens from these six southern samples are immatures 10.5 to 20.1 mm in length; they were collected in June and September.

Water depths at all NMFS collecting sites range from 29 to 159 m. Bottom sediments at these localities consist of a variety of types ranging from fine-textured clays to gravel. Most of the samples, however, come from intermediate types of sediment: silt-clay (38%), glacial till (27%), and sand (22%). Only a small percentage of samples represent other bottom types: silt-sand (7%), gravel (4%), and clay (1%).

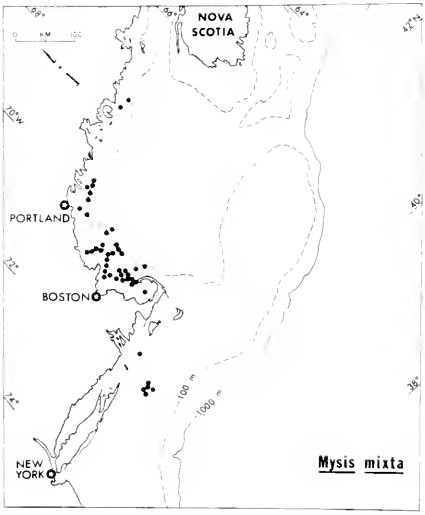


FIGURE 14.—Geographic distribution of *Mysis mixta* based on specimens in the collection at the NMFS Biological Laboratory, Woods Hole.

The occurrence of specimens at the surface (*Albatross IV*, cruise 66-14, station 24) and in several samples between middepths and surface (*Albatross IV*, cruise 66-14, stations 35, 36, 39, and 40), as well as those taken on the sea bottom, indicates a diurnal vertical migration. Specimens at the surface were collected at 0300 hr and midwater samples were taken between 2400 and 0900 hr. This implies inhabitance of upper waters during hours of darkness and a bottom habitat in the daytime.

Sex Ratio

The NMFS collection contains 119 females but only 2 males, which is a ratio of 0.017 male to 1 female. Whether males occur in a habitat different from females, or are actually so much less abundant than females, is unknown. This is the only species in the collection having such a grossly unbalanced sex ratio.

Body Size

Body lengths range from 5.0 to 25.0 mm. Immature specimens are a common component, accounting for 69% of all specimens caught. Many of these immature specimens are large (15 to 20 mm). Also noteworthy is that many large males and females do not possess fully developed secondary sex characteristics. A summary of body length measurements by months and stage of development is listed in Table 7.

TABLE 7.—Means and ranges of body length of *Mysis mixta* by months and stages of development.

Month	Body length			
	Immatures		Adults	
	Mean	Range	Mean	Range
	mm	*mm*	*mm*	*mm*
May	6.9	5.0- 8.5	20.6	19.0-21.3
Jun.	10.5	--	--	--
Aug	13.6	10.3-20.0	22.0	20.0-24.2
Sept.	18.8	17.2-20.1	--	--
Oct.	16.7	14.0-20.0	23.4	22.0-25.0

Length of Life and Spawning

Although Smith (1879) suggested this species might be an annual that spawns during the winter, our analysis of the maturity status and size frequency data of the NMFS specimens leads us to believe that *M. mixta* has a 2-year life span and spawns in winter or early spring.

Our material is scanty for purposes of determining length of life, but the data do indicate a clear trend in growth and development (Table 7). The specimens reveal two definite age groups in both spring and fall. In May the immatures average 6.9 mm and the adults 20.6 mm. In October the immatures average 16.7 mm and the adults 23.4 mm. Juveniles increase in length at a rate of about 2 mm per month until winter when the growth rate slows appreciably. They mature and spawn in the winter or early spring.

None of the NMFS specimens are ovigerous. The only indication of spawning season is the presence of small (5.3 to 6.3 mm) specimens in May, which suggests a late winter or early spring spawning.

Mysis stenolepis S. I. Smith, 1873

This large American mysid is distributed in coastal waters of northeastern North America from the Gulf of St. Lawrence to New Jersey. Though very common to this region it is represented by only a few specimens in the NMFS collection because biological samples were rarely collected in the intertidal and shallow subtidal zones.

The NMFS collection contains 13 specimens from six samples (Figure 15; Burns and Wigley, Table 16), all from a rather small area off eastern Massachusetts and eastern Rhode Island. The specimens were collected with a dip net along shore in water depths of about 1 m. Four samples were taken in beds of *Zostera* or algae, and one sample was from a sandy bottom (bottom type for the other sample is unknown).

M. stenolepis is one of the largest shallowwater species in the NMFS collection. Body lengths of these specimens range from 13.0 to 26.0 mm, and average 20.4 mm.

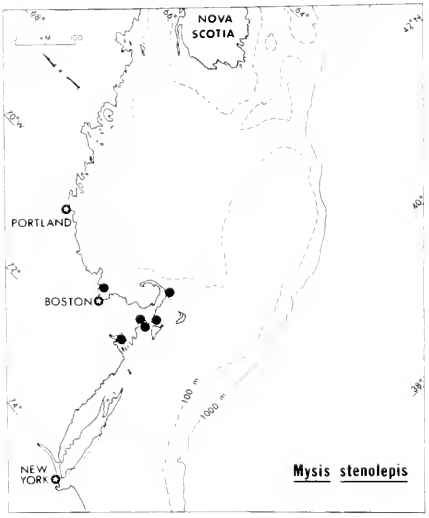


FIGURE 15.—Geographic distribution of *Mysis stenolepis* based on specimens in the collection at the NMFS Biological Laboratory, Woods Hole.

According to Smith (1879) the life span of this species is one year. Their life cycle is as follows: (1) adults spawn in winter and early spring, (2) the young appear in late spring and summer, and (3) they mature in the fall and winter. Material in the NMFS collection substantiates this life-cycle plan. Body lengths of specimens, by months, are:

Month collected	Average body length (*mm*)	Stage of maturity
September	14.2	Immature
October	20.0	Mature
November	24.2	Mature
February	25.3	Mature
March	25.0	Mature
April	26.0	Mature

Three larvigerous specimens collected in February and March range in size from 25.0 to 25.5 mm.

Fecundity of this species is very high compared with other east coast mysids. An ovigerous-larvigerous specimen 25.0 mm long, collected in late February, held 94 eggs and 50 stage III larvae in the brood pouch. Average diameter of the eggs is 0.4 mm and average length of the larvae is 1.4 mm. A 25.5-mm long larvigerous female from the same sample was carrying 188 stage IV larvae; their average length is 1.1 mm. A 25.0-mm larvigerous female collected in March was carrying 171 stage VII larvae; their average length is 1.86 mm.

A rather high water temperature was noted when the September 4, 1961, sample of *M. stenolepis* was collected. The temperature was 23.9° C at a depth of 1 m in an eelgrass bed in Waquoit Bay, Falmouth, Mass. This is the highest temperature recorded for the NMFS samples of this species.

Praunus flexuosus (O. F. Müller, 1776)

This species is very common in shallow coastal waters of Great Britain and along the northern coast of France, Holland, and southern Scandinavia (Tattersall and Tattersall, 1951). In 1960 it was discovered in the harbor at Barnstable,

Mass., (Wigley, 1963) the first report of its occurrence outside the north European area. It has also been collected in large numbers from the coast of New Hampshire by Dr. William F. Black (personal communication), and from the Penobscot River (Maine) estuary by Haefner (1969).

The NMFS collection contains 15 specimens from five samples (Figure 16; Burns and Wigley, Table 17), including four specimens from the original seven taken at Barnstable in 1960.

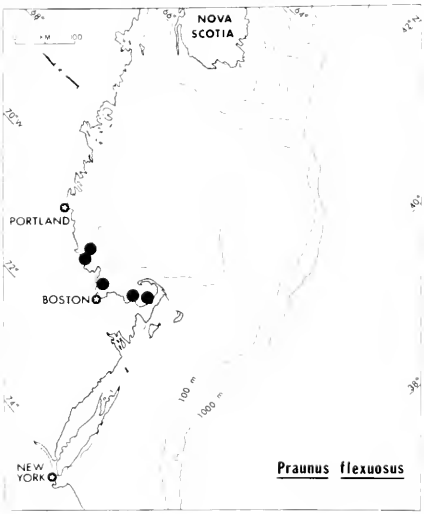


FIGURE 16.—Geographic distribution of *Praunus flexuosus* based on specimens at the NMFS Biological Laboratory, Woods Hole.

[The other three specimens from this collection were sent to Dr. Olive S. Tattersall, who very kindly examined them and verified the identification (Burns and Wigley, Table 17; footnote 2).] The other samples are also from coastal areas north of Cape Cod, Mass. These were collected along shore at Manomet, Mass.; in the harbor at Nahant, Mass. (by Dr. Nathan W. Riser); at Rye Harbor, N.H. (by Mr. John A. Lindsay); and from a tide pool on Appledore Island, Isle of Shoals, N.H. (by Mr. Stephen Tonjes).

This is a shallowwater species commonly found in tide pools and associated with algae or *Zostera*. All samples in the NMFS collection are from nearshore localities at depths of 5 m or less. The majority of samples were collected by means of a dip net.

Size range of all NMFS specimens is 15.0 to 28.0 mm; males are 15.5 to 19.0 mm and females 16.5 to 28.0 mm. Only three immature specimens, ranging in length from 15.0 to 16.5 mm, are represented in the collection. Sex ratio of the 12 adult specimens is one male to one female.

The spawning season for this species in New England waters, based on the one ovigerous and two larvigerous females in the collection, is at least April through November. It may, however, spawn in this region throughout the year, as it does in Europe. The 25.0-mm female from Barnstable, Mass., held 44 eggs in the brood pouch. The 21.0-mm female from Isle of Shoals, N.H., held 39 stage V larvae in the brood pouch. Although the larvigerous female in the Rye Harbor sample contained 19 larvae, the oostegites were separated and it appeared to be an incomplete clutch.

When this species was discovered in North America in 1960, the question arose whether it was a recent immigrant from Europe, or whether it had inhabited this region for hundreds of years but had been overlooked. After the first capture of only seven specimens most considered it a rare species with only a local distribution in New England. Additional information obtained since 1960, however, indicates it is rather widely distributed between Maine and Cape Cod, Mass., and that it is abundant in the Maine-New Hampshire region. In view of this, and considering the intensive collecting in shallow coastal waters of New England by A. E. Verrill, S. I. Smith, W. Stimpson, and numerous other scientists during the latter half of the nineteenth century, it is our conjecture that *P. flexuosus* is a comparatively new addition to the New England fauna. Possibly it was transported from Europe to a New England port, such as Boston or Portsmouth, N.H., among fouling organisms on the

bottoms of ships during World War II when convoys of merchant ships were making frequent and rather regular transoceanic voyages.

Neomysis americana (S. I. Smith, 1873)

Geographic Distribution

N. americana is the most common mysid inhabiting the northeastern coastal waters of the United States and undoubtedly the most abundant mysid in the western North Atlantic Ocean. It is strictly a North American species, having been reported only from the Gulf of St. Lawrence south to Virginia. It is much more abundant and widely distributed between Virginia and New England than in the northern part of its range.

The NMFS collection originally contained over 2 million specimens of this species—more numerous than any other mysid in the collection, but for purposes of analysis the larger lots were subsampled. Subsamples totaling 8,451 specimens from 168 samples (Figure 17; Burns and Wigley, Table 18) were examined. The geographic distribution of specimens in this collection ranges from off Nova Scotia, near the mouth of the Bay of Fundy, south to Chesapeake Bay.

Most of the specimens are from two regions (Figure 17): (1) eastern Georges Bank to Rhode Island, and (2) from northern New Jersey to Chesapeake Bay. The gap in distribution between these two areas (off Long Island) appears to be more pronounced in offshore waters than inshore. Bigelow and Sears (1939) also encountered a broad hiatus in the occurrence of this species in the offshore waters of eastern Long Island. Yet, inshore in the New York region it has been reported from Great South Bay, Long Island (Smith, 1879) and from Long Island Sound (Verrill, Smith, and Harger, 1873; Smith, 1879; and Richards and Riley, 1967). The only record from offshore Long Island known to us is that reported by Grice and Hart (see footnote 3) which indicated the presence of this species in two plankton samples taken at station 13 located at lat 40°44′ N and long 71°41′ W (water depth, 64 m). The above records indicate merely a restricted occurrence or low abundance in the offshore New York region, not a complete break in distribution.

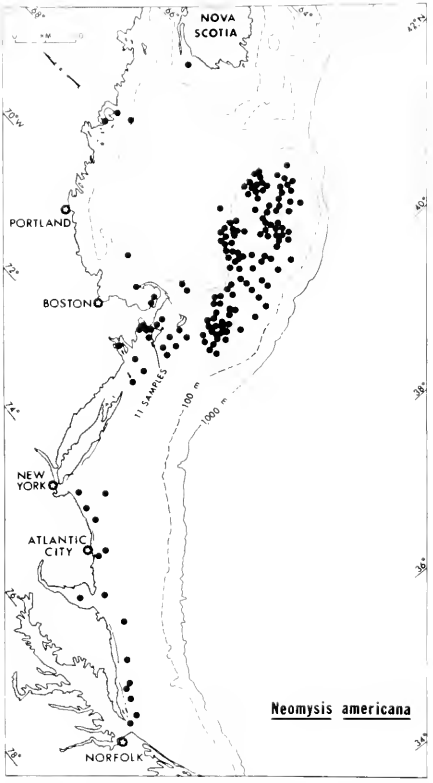


FIGURE 17.—Geographic distribution of *Neomysis americana* based on specimens in the collection at the NMFS Biological Laboratory, Woods Hole.

Samples from the Georges Bank area during summer and winter revealed a similar distribution of *N. americana* in both seasons. The species was present over most of the bank, with highest concentrations in the central part, the same area where Whiteley (1948) also found them to be most abundant.

Bathymetric Distribution

N. americana is a shallowwater species most commonly reported from the intertidal zone to depths of 60 m. However, it appears to inhabit somewhat deeper water in the Georges Bank region as indicated by the records of occurrence in Figure 17. Whiteley (1948) reported it from a number of plankton samples taken at stations where the water depth was 75 m, but at very few localities where depths were greater than 100 m. Greatest depth reported for this species is 214 m (Wigley, 1964).

Depth range for the samples in the NMFS collection is 1 to 232 m. Frequency distribution for these samples is listed in Table 8. This species is common from the intertidal zone out to 90 m but is most abundant at depths between 30 and 60 m.

TABLE 8.—Bathymetric distribution of *Neomysis americana*, based on the NMFS collection.

Water depth	Samples	Specimens
m	*Number*	*Number*
0- 9	5	147
10- 19	14	139
20- 29	20	361
30- 39	19	3,289
40- 49	38	1,998
50- 59	32	1,619
60- 69	13	299
70- 79	12	308
80- 89	4	220
90- 99	2	2
100-109	3	6
150-159	1	3
230-239	1	1
Unclassified	4	59
Total	168	8,451

N. americana undertakes regular vertical migrations between the sea bottom and the upper water layers. Light intensity is the primary controlling element to which the mysids are responding. They move to deeper, darker regions during daytime and upward toward the surface at night (Hurlbut, 1957; Herman, 1963). The NMFS samples provide very little information on this aspect other than to substantiate probable vertical migration in shallow water. At depths of less than 50 m on Georges Bank this species is more common in bottom samples collected during the daytime than at night.

Spawning

Though spawning of coastal populations of *N. americana* takes place throughout the year, it is much more intensive during the warmer months of April through October (Smith, 1879; Fish, 1925; Cowles, 1930; Herman, 1963; Hopkins, 1965). The Georges Bank population was reported by Whiteley (1948) to spawn in the spring. Specimens in the NMFS collection indicate spawning of Georges Bank stocks from March through October and possible spawning in all months of the year. There appear to be two major spawning periods, one in the spring (March through June) and another in the late summer and fall (August through October). Of the ovigerous or largiverous specimens present in samples collected every month from March to October the largest numbers occurred in March through June and August through October. Immature specimens were particularly numerous in August and December. The small number of immature specimens collected in late winter and early spring may indicate occasional small-scale spawning in winter.

Two distinct size groups (summer generation and overwintering generation) of spawning females are discernable; one group spawns in the spring, the other in the fall (Figure 18). Spring spawners have an average length of 11 to 12 mm, and produce a clutch containing about 26 eggs. Fall spawners have an average length of 6 to 8 mm, and their clutch contains only about six eggs. Additional information about these two generations is given below.

Eggs are spherical, 0.38 to 0.42 mm in diameter, in both the summer and overwintering generations.

Size of the larvae varies according to their stage of development. Average lengths in millimeters for the following stages are: stages I and II—0.39, stage III—0.55, stage IV—0.85, stage V—0.96, stage VI—1.15, and stage VII—1.34.

Sex Ratio

The NMFS collection contains 1,571 males and 1,669 females; the ratio is 0.94 male to 1 female.

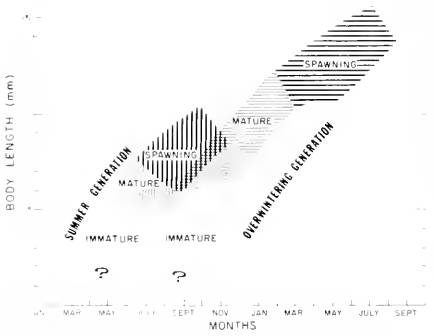


FIGURE 18.—Schematic diagram of the age-size-maturity composition of *Neomysis americana* populations from offshore New England.

Length of Life

The Georges Bank population of *N. americana* appears to consist of two generations: (1) a short-lived summer generation and (2) a long-lived overwintering generation (Table 9, Figure 18). The summer generation stems from eggs that hatch in late winter to late spring. They grow rapidly and mature in late summer and autumn. Length of life of this generation is estimated to be 6 to 10 months. They are the progeny of the overwintering group, the dominant group in the offshore New England area. The overwintering generation originates from eggs that hatch during the summer and autumn, and perhaps even from late spring eggs. They grow at nearly the same rate as the summer generation but do not reach maturity until the following spring. Thus, they are substantially larger than the summer generation. Adults of the overwintering generation are 10 to 15 mm long, compared with 6 to 10 mm lengths for the summer generation adults. Estimated length of life of this overwintering group is 10 to 14 months.

TABLE 9.—Range in body length of the (1) summer and (2) overwintering generations of *Neomysis americana*, by sexes and periods.

Period	Range in length			
			Females	
	Immatures[1]	Males	All specimens	Ovigerous and larvigerous
	mm	*mm*	*mm*	*mm*
Summer generation				
May -June	3.0- 6.8	5.9- 7.4	6.0- 7.4	--
July -Aug	--	5.0-(9.0)	5.1-(9.0)	6.0- 8.9
Sept.-Oct.	--	5.7-10.0	6.0- 9.6	6.6- 8.3
Overwintering generation				
Sept.-Oct	3.0- 7.0	--	--	--
Nov.-Dec.	3.1- 7.4	5.5- 9.6	5.6- 9.6	--
Jan. -Feb.	8.3-10.5	10.6-12.9	11.5-14.7	--
Mar.-Apr.	6.0-10.9	9.3-12.1	9.3-13.3	10.7-12.4
May -June	--	9.5-14.0	9.5-14.0	11.4-14.0
July -Aug	--	ca (9)-13.0	ca (9)-(14.0)	--

[1] Immatures less than 3 to 4 mm length usually passed through the meshes of the sampling and processing equipment

Relation to Bottom Sediments

Although *N. americana* make daily excursions from the sea bottom to upper water levels (see Bathymetric Distribution), a substantial amount of their time is spent on bottom, and they appear to be selective in the type of bottom they inhabit. The bottom type with which they are most commonly associated is sand (Table 10). Kinds of sands they inhabit, in decreasing order of importance are: fine, medium, and coarse. One explanation for the scarcity of *N. americana* in the middle and outer shelf areas south of Rhode Island and New York may be unsuitable sediments. The bottom over much of this area is blanketed with silty sands and sandy silt, whereas on Georges Bank and much

TABLE 10.—Frequency of occurrence of *Neomysis americana* in various types of bottom sediments, based on the NMFS collection.

Bottom type	Samples	Specimens
	Number	*Number*
Rock-gravel	5	262
Gravel-sand	10	37
Glacial till	0	0
Shell-sand	4	51
Sand	131	7,987
Silt sand	7	30
Silt-clay	7	25
Unclassified	4	59
Total	168	8,451

of the nearshore coastal areas where *N. americana* is common, the bottom types are predominantly sands with low silt content (Wigley, 1961; Uchupi, 1963).

Relation to Water Temperature

This mysid is eurythermic and the extremes of temperature in shallow New England waters (0° to over 20° C), in shallow portions of Georges Bank (2°-18° C), and in the vicinity of the Chesapeake Bay (over 25° C), do not appear to inhibit survival of this species. Reproduction and other life processes, however, are affected by temperature. Also, the sequence, timing, or duration of temperature regimes may be important. For example, in the offshore region south of Rhode Island and Long Island, N.Y., where there is a low abundance of this species, the presence of a layer of cold bottom water (the so-called "cold bubble") may have a pronounced influence in repelling immigrants or retarding reproduction.

Tribe HETEROMYSINI

Heteromysis formosa S. I. Smith, 1873

H. formosa is an amphi-Atlantic species that has been reported in the eastern Atlantic from the northern coast of France, British Isles, and Norway. In the western Atlantic it is known to occur along the eastern and southern coasts of the United States from Maine to the Gulf of Mexico. All except three of the western Atlantic records are from the northeastern sector, between Maine and New Jersey. The three southern records are all from relatively deepwater (18 to 227 m) localities. Specimens from 18 m were collected by Brattegard (1969) off Fort Pierce, Fla. The other two records, reported by Tattersall (1951), are based on collections of the research vessel *Albatross* at a depth of 227 m off the coast of North Carolina (lat 34°38′ N, long 75°31′ W) and in eastern Gulf of Mexico (lat 28°36′ N, long 85°34′ W) at a depth of 203 m. (One additional deepwater sample was collected in the northern region by the research vessel *Fish Hawk* at station 917, located south of Martha's Vineyard, Mass., at lat 40°22′ N, long 70°12′ W at a depth of 81 m.)

The NMFS collection contains 72 specimens from 15 samples (Figure 19; Burns and Wigley, Table 19). The geographic distribution of these

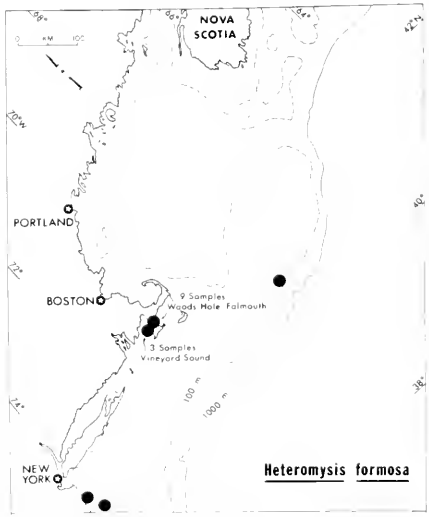


FIGURE 19.—Geographic distribution of *Heteromysis formosa* based on specimens in the collection at the NMFS Biological Laboratory, Woods Hole.

samples extends from southwestern Georges Bank (1 sample) and southern Massachusetts (12 samples) to northern New Jersey (2 samples).

Although the bathymetric range for the NMFS samples is 2 to 84 m, only one sample containing a single specimen was taken at 84 m. All others were collected at depths of 26 m or less. This species customarily inhabits the shallow (1-20 m) inshore areas, such as harbors, bays, and estuaries, where it is much more common than on the outer portion of the continental shelf. The presence of only a single specimen in the NMFS collection from moderately deep water on the outer continental shelf, in contrast to the 71 specimens from inshore locations, illus-

trates the relative scarcity of this species offshore.

Based on NMFS samples, spawning takes place from June to September. Ovigerous or larvigerous females are 5.0 to 8.0 mm long and the number of young per brood is 13 to 15. The average diameter of eggs is 0.4 mm; the length of stage VI larvae is 1.0 mm and of stage VII larvae is 1.7 mm.

The NMFS collection contains 27 males and 31 females, a ratio of 0.9 male to 1 female.

Immature specimens within the size range of 3.4 to 4.5 mm are present in samples from October through January. Body lengths of adults range from 4.7 to 8.9 mm.

This species was collected from a variety of different bottom types (gravel, sand, coarse sand, glacial till, and silty sand). Apparently it has no special affinity for any one kind of sediment, but appears to be more commonly associated with coarse-textured sediments. Members of this species congregate in dead shells of bivalves such as *Mercenaria* and *Spisula*.

Sizes of adult specimens from coastal areas are approximately 5 to 9 mm, whereas the offshore specimens reportedly reach lengths of 15 mm. Owing in part to this difference in size, and partly to their deepwater habitat, Tattersall (1951) considered the possibility that the large offshore specimens collected by the research vessels *Albatross* and *Fish Hawk* might represent a new species closely related to *H. formosa*. He concluded, however, that both groups were similar and only one species was represented.

The deepwater specimen in the NMFS collection is a female only 6.5 mm long. A morphological comparison of this specimen with inshore specimens revealed no major differences that we could detect.

SUMMARY

GENERAL

The principal biological and ecological characteristics for each of the 19 species in the NMFS collection are summarized in abbreviated form in Table 11. This tabulation provides a condensed comparison of one species with another within the NMFS collection and can be used for comparing NMFS information with data from other sources.

TAXONOMIC AFFINITY AND ENDEMISM

The taxonomic affinities of mysids in the NMFS collection are most closely allied with the boreal and subarctic species in the North Atlantic; however, a high degree of endemism is evident.

Nine species having an amphi-Atlantic distribution are: *Eucopia grimaldii*, *Boreomysis tridens*, *Erythrops erythrophthalma*, *Meterythrops robusta*, *Pseudomma affine*, *Amblyops abbreviata*, *Mysis mixta*, *Pranus flexuosus*, and *Heteromysis formosa*. The eight species that are indigenous to the western North Atlantic are: *Bowmaniella portoricensis*, *Hypererythrops caribbaea*, *Pseudomma* sp., *Bathymysis renoculata*, *Mysidopsis bigelowi*, *M. furca*, *Mysis stenolepis*, and *Neomysis americana*. These indigenous species are all inhabitants of warm-temperate to tropical waters. Only one species in the NMFS collection (*Eucopia grimaldii*) is cosmopolitan. Four species (*Eucopia grimaldii*, *Anchialina typica*, *Meterythrops robusta*, and *Amblyops abbreviata*) occur in the Pacific Ocean as well as in the western Atlantic. One species (*Promysis atlantica*) occurs in the South Atlantic and North Atlantic Oceans.

GEOGRAPHIC DISTRIBUTION

The geographic distribution of species represented in the NMFS collection differs in scope from single records (of which there are three) to wide-ranging multiple records. *Mysidopsis bigelowi* has the greatest range, extending from northern Georges Bank southward to northern Florida. *Neomysis americana* has a moderate range, extending from northern Gulf of Maine south to Chesapeake Bay. *Bowmaniella portoricensis* and *Promysis atlantica* have widespread ranges in the southern area, with distributions extending from Virginia to Florida. All remaining species were collected within rather limited geographic areas along the eastern coast of the United States, mostly off New England.

TABLE 11.—Summary of biological and ecological information, by species, pertaining only to mysids in the NMFS collection.

Species	Geographic distribution	Bathymetric range	Bottom type	Body length		Spawning season	Number of eggs per clutch[1]
				Range	Smallest adult		
		m		mm	mm		
Eucopia grimaldii	Slope off southern New England	700	(Silt-clay)	32.0	32.0	--	--
Boreomysis tridens	Slope off southern New England	402	Silt-sand	15.0-26.0	26.0	--	--
Bowmaniella portoricensis	Inner shelf Virginia to Florida	9- 56	Sand	3.1-10.0	6.1	Spring and summer	30
Inchialina typica	Inner shelf South Carolina to northern Florida	32- 38	Sand	4.5- 5.0	4.5	--	--
Erythrops erythrophthalma	Inner and outer shelf and upper slope off New England	18-421	Sand	3.0- 9.6	4.3	(May-Oct.)[2] Aug. and Sept.	15
Meterythrops robusta	Gulf of Maine	64-150	Gravel-sand	6.6-12.0	8.5	(Possibly spring)[2]	--
Hypererythrops caribbaea	Outer shelf off New England	168-179	Sand	5.5-11.0	9.5	Aug.	--
Pseudomma affine	Outer shelf and upper slope off New England	146-329	Silt-sand	4.0-13.1	7.3	July-Dec.[2]	11
Pseudomma sp.	Outer shelf off New England	--	Silt-sand	--	--	--	--
Amblyops abbreviata	Gulf of Maine	183-329	Silt-clay	4.7-15.0	10.0	Dec.	29
Bathymysis renoculata	Slope off southern New England	220-366	Silt-clay	4.0-16.2	13.0	(Possibly spring)[2]	--
Mysidopsis bigelowi	Inner and outer shelf, Georges Bank to Florida	13-179	Sand	2.4- 6.6	3.5	(Possibly June-Oct.)[2]	--
Mysidopsis furca	Inner shelf off South Carolina	22	Sand	4.2	4.2	--	--
Promysis atlantica	Inner shelf, Virginia to Florida	8- 26	Sand	4.5- 5.0	4.5	--	--
Mysis mixta	Inner shelf off New England	29-159	Various	5.0-25.0	19.0	(Possibly winter or early spring)[2]	--
Mysis stenolepis	Shores of southern Massachusetts and Rhode Island	1	Sand and *Zostera*	13.0-26.0	20.0	Feb. and Mar.	188
Praunus flexuosus	Shores of New Hampshire and eastern Massachusetts	1	Various	15.5-28.0	15.5	Apr.-Nov.	44
Neomysis americana	Inner and outer shelf off New England, inner shelf New Jersey to Virginia	1-232	Sand	3.0-14.7	5.5	Mar.-Oct. (possibly also in winter)	36 [3] 26 [4]
Heteromysis formosa	Inner and outer shelf, Massachusetts to New Jersey	2- 84	Various	3.4- 8.9	4.6	June-Sept.	15

[1] A large proportion of ovigerous females had an incomplete clutch. The values given here refer only to those with a full complement of eggs.
[2] Deduced from the presence of immature specimens at a somewhat later season.
[3] Clutch size of the summer generation.
[4] Clutch size of the overwintering generation.

The presence of 15 species in the New England region (Table 12), compared with only 3 species in the Middle Atlantic and 5 species in the Southern area, is due, in part, to more intensive sampling in the New England waters. However, the recovery of five species in samples from the southeastern coast of the United States where the sampling was sparse, indicates a relatively diverse mysid fauna inhabits that region. Thorough sampling will undoubtedly disclose a number of additional species (new species plus new records for presently recognized species) in all sections of the coast, though the Middle Atlantic region can be expected to contain the fewest species of mysids.

TABLE 12.—Geographic classification of species, based on the NMFS collection.

New England (Nova Scotia south to Hudson Canyon)	Middle Atlantic (Hudson Canyon south to northern Virginia)	Southern (Northern Virginia south to Florida)
Eucopia grimaldii		*Bowmaniella portoricensis*
Boreomysis tridens		*Inchialina typica*
Erythrops erythrophthalma		
Meterythrops robusta		
Hypererythrops caribbaea		
Pseudomma affine		
Pseudomma sp.		
Amblyops abbreviata		
Bathymysis renoculata		
Mysidopsis bigelowi	*Mysidopsis bigelowi*	*Mysidopsis bigelowi*
Mysis mixta		*Mysidopsis furca*
Mysis stenolepis		*Promysis atlantica*
Praunus flexuosus		
Neomysis americana	*Neomysis americana*	
Heteromysis formosa	*Heteromysis formosa*	

BATHYMETRIC DISTRIBUTION

The overall bathymetric range at which NMFS mysids were collected is from 1 to 700 m (Table 11). In general all depth zones are rather evenly represented without a preponderance in any one zone. In Table 13 the species are listed under five categories based on the water depths from which they were most frequently caught. Two species were found only in the intertidal zone. Five species are typically

TABLE 13.—Bathymetric classification of species, based on the NMFS collection.

1. Shore Species (occur in the intertidal zone, minimum and maximum depth 0 and 1 m):
   Mysis stenolepis
   Praunus flexuosus
2. Shallow Shelf Species (occur predominantly at depths less than 50 m, minimum and maximum depth 2 and 84 m):
   Bowmaniella portoricensis
   Anchialina typica
   Mysidopsis furca
   Promysis atlantica
   Heteromysis formosa
3. Eurybathic Shelf Species (occur over a broad range of depth on the continental shelf, minimum and maximum depth 1 and 421 m):
   Erythrops erythrophthalma
   Meterythrops robusta
   Mysidopsis bigelowi
   Mysis mixta
   Neomysis americana
   Pseudomma sp.
4. Deep Shelf and Upper Slope Species (occur on the continental slope and outer shelf, minimum and maximum depth 98 and 329 m)
   Hypererythrops caribbaea
   Pseudomma affine
   Amblyops abbreviata
5. Slope Species (occur predominantly on the continental slope, minimum and maximum depth 220 and 700 m):
   Eucopia grimaldii
   Boreomysis tridens
   Bathymysis renoculata

Shallow Shelf (less than 50 m) inhabitants. *Hetermoysis formosa* is included in this category even though one specimen was taken at a depth of 84 m. This is the only New England species in this bathymetric category; all other Shallow Shelf species are warm-water forms collected in the southern region. Six species are listed under the heading "Eurybathic Shelf Species." They were each taken over a broad depth range (for example, *Neomysis americana*, 1-232 m) on the continental shelf and occasionally on the upper continental slope. Three species that live along the outer margin of the continental shelf are listed under the category "Deep Shelf and Upper Slope Species." Depth range for these species is 98 to 329 m. Three species were taken at depths beyond the outer margin of the continental shelf, from 220 to 700 m. They are listed under the category "Slope Species."

SPAWNING

Information pertaining to the spawning seasons of 13 mysid species in the NMFS collection is summarized in Table 11. Direct information based on the capture of ovigerous or larvigerous females is the most convincing evidence; this was obtained for eight species. Additionally, indirect evidence from catch records of immature specimens provides clues to possible spawning seasons of seven species, including four species for which direct evidence is lacking.

Spawning of most species for which information is available takes place during the warmer months—May through October. Species that spawn in this season are: *Bowmaniella portoricensis*, *Erythrops erythrophthalma*, *Hypererythrops caribbaea*, *Pseudomma affine*, *Amblyops abbreviata*, *Praunus flexuosus*, *Neomysis americana*, *Heteromysis formosa*, and possibly *Bathymysis renoculata* and *Mysidopsis bigelowi*. One species, *Neomysis americana*, probably spawns in all seasons of the year with maximum production in spring. *Amblyops abbreviata* and *Pseudomma affine* spawn in winter and summer; *Meterythrops robusta*, *Mysis mixta*, and *M. stenolepis* probably spawn in winter or early spring.

The number of eggs or larvae per clutch was counted for eight species. Although the average number per clutch for different species ranges from 6 to 188, these extremes are rare. For most species the average brood contains between 11 and 30; exceptionally small clutches (6 eggs) were produced only by the summer generation of *Neomysis americana*. Unusually large clutches (average of 188 eggs) were typical for one species, *Mysis stenolepis*. A moderately large number of eggs (average of 44) was produced by *Praunus flexuosus*. Both of the latter species are relatively large inshore inhabitants. Small species commonly brood as many eggs as moderately large species; within a species, however, the smaller specimens have fewer eggs than large specimens. The diameter of eggs of ovigerous mysids in the collection was surprisingly uniform. Both large and small species produced eggs that were approximately 0.4 mm in diameter.

BODY SIZE

The smallest and largest specimens (excluding larvae) in the NMFS collection are 2.4 and 32.0 mm in body length. Body lengths were measured for 18 of the 19 species represented

in the collection. (Specimens of *Pseudomma* sp. have not yet been measured.) They have been classified as small, medium, or large. Two criteria were used for determining the appropriate size category: (1) the maximum length of specimens of each species represented in the collection and (2) the length of the smallest adult of each species.

Small species are those with a maximum length of 6.6 mm or less and with the smallest adult 4.5 mm or less. There are four species in this category: *Anchialina typica*, *Mysidopsis bigelowi*, *M. furca*, and *Promysis atlantica*. *Mysidopsis bigelowi* is the smallest species encountered; it matures at a body length of 3.5 mm.

Medium size species are those having a maximum length between 8.9 and 16.2 mm and with the smallest adult 4.6 to 13.0 mm long. There are nine species in this category: *Bowmaniella portoricensis*, *Erythrops erythrophthalma*, *Meterythrops robusta*, *Hypererythrops caribbaea*, *Pseudomma affine*, *Amblyops abbreviata*, *Bathymysis renoculata*, *Neomysis americana*, and *Heteromysis formosa*.

Large species are those with a maximum length of 25.0 mm or more and with the smallest adult more than 15.0 mm long. There are five species in this category: *Eucopia grimaldii*, *Boreomysis tridens*, *Mysis mixta*, *M. stenolepis*, and *Praunus flexuosus*.

RELATION TO BOTTOM SEDIMENTS

A large majority of mysid species in the NMFS collection live on bottom sediments composed of sand or silty sand. They were least abundant and seldom encountered in gravel and rocky areas. (*Eucopia grimaldii* is excluded from this discussion of mysids in relation to bottom sediments, because it is a bathypelagic species.) Eleven of the 18 benthic species were most commonly associated with sand and silty sand. The sand-dwelling species are: *Bowmaniella portoricensis*, *Anchialina typica*, *Erythrops erythrophthalma*, *Hypererythrops caribbaea*, *Mysidopsis bigelowi*, *M. furca*, *Promysis atlantica*, and *Neomysis americana*. The two most common species are both included with the sand-dwelling inhabitants, but there are significant differences in the habitats they occupy. *Neomysis americana* are occasionally taken on silty sand bottoms, but typically inhabit sand sediments that are silt free or contain very little silt. Conversely, *Erythrops erythrophthalma* have their center of abundance in areas of sand sediments that contain small to moderate quantities of silt. However, the silt content of the sands they occupy is usually insufficient to classify them as silty sands according to the standard classification established by Shepard (1954). Silty sand inhabitants are: *Boreomysis tridens*, *Pseudomma affine*, and *Pseudomma* sp. The only species that is frequently associated with gravels and other coarse substrates is *Meterythrops robusta*.

Species associated with fine-textured sediments or with various types of bottom materials were usually less abundant and present at fewer localities than the species listed above. Species that were associated with silt-clays are: *Amblyops abbreviata* and *Bathymysis renoculata*. Both species are deepwater inhabitants. Their overall depth range is 183 to 366 m. Fine-grained sediments blanket a large portion of the sea floor at these depths. Species that were found occupying a wide variety of different kinds of bottom sediment types are: *Mysis mixta*, *Praunus flexuosus*, and *Heteromysis formosa*. These are shallowwater species and the most common bottom types they inhabited were: sand, gravel, silt-clay, glacial till, algae, and eelgrass (*Zostera*).

CO-OCCURRING SPECIES

The catch records reveal a high incidence of co-occurrence of the Atlantic coast mysids. Listed in Table 14 are 15 species, 79% of the total number of species collected, taken in the same sample with one or more other species of mysids. The presence of different species of mysids in dredges, trawls, ring nets, and similar sampling instruments that are towed along the ocean bottom for relatively long distances (hundred meters to several kilometers) reveals a reasonably close spatial occurrence. Unfortunately, the spatial separation between specimens of the different species in such samples prior to

collection are unknown. It was especially interesting to find two species in the same grab sample (Campbell sampler or Smith-McIntyre sampler), for example, *Bowmaniella portoricensis* with *Anchialina typica* or *Mysidopsis furca*. This is good evidence that within an area of 0.48 m² of sea bottom *B. portoricensis* lives with *A. typica* or *M. furca*. Also, *Neomysis americana* and *Mysidopsis bigelowi* were caught in the same grab (Smith-McIntyre sampler) samples, but in this case both species were taken from an area of bottom only 0.1 m². These examples, of course, do not mean that these species are competitors. They are strong indicators, however, of close habitation and possible competition for space or other living requirements.

TABLE 14.—A list of co-occurring species. Species in column B were present in one or more samples with the corresponding species listed in column A.

A	B
Bowmaniella portoricensis	*Anchialina typica* *Mysidopsis furca*
Anchialina typica	*Bowmaniella portoricensis*
Erythrops erythrophthalma	*Hypererythrops caribbaea* *Pseudomma affine* *Pseudomma* sp *Mysidopsis bigelowi* *Mysis mixta* *Neomysis americana* *Heteromysis formosa*
Pseudomma affine	*Erythrops erythrophthalma* *Hypererythrops caribbaea* *Amblyops abbreviata*
Pseudomma sp	*Erythrops erythrophthalma*
Amblyops abbreviata	*Pseudomma affine*
Mysidopsis bigelowi	*Erythrops erythrophthalma* *Hypererythrops caribbaea* *Neomysis americana*
Meterythrops robusta	*Mysis mixta*
Hypererythrops caribbaea	*Erythrops erythrophthalma* *Pseudomma affine*
Mysidopsis furca	*Bowmaniella portoricensis*
Mysis mixta	*Erythrops erythrophthalma* *Meterythrops robusta* *Neomysis americana*
Mysis stenolepis	*Praunus flexuosus*
Praunus flexuosus	*Mysis stenolepis*
Neomysis americana	*Erythrops erythrophthalma* *Hypererythrops caribbaea* *Mysidopsis bigelowi* *Mysis mixta*
Heteromysis formosa	*Erythrops erythrophthalma*

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ESCAPEMENT LEVELS AND PRODUCTIVITY OF THE NUSHAGAK SOCKEYE SALMON RUN FROM 1908 TO 1966[1]

OLE A. MATHISEN[2]

ABSTRACT

Since the inception of a commercial fishery for sockeye salmon in the Nushagak District, Bristol Bay, Alaska, the annual yields have followed a definite pattern. Catches increased during a relatively short development phase of the fishery, then stabilized for some years and then declined in two steps separated by periods of relative stability.

For years the cause of the decline had been thought to be overfishing, and various measures of curtailment had been placed upon the fishing industry.

Evidence is presented in this paper that the average escapement or the potential egg deposition remained about the same during each of three periods (1908-1919, 1925-1945, and 1946-1966); hence the diminution in the runs was due not to lack of spawners but to a decline in the rate of return per spawner.

So that the cause or causes of the present low reproductive potential can be ascertained, the effects of fishing on the stocks of salmon must be examined. Besides removing part of the run, the yearly commercial fishing operation may have altered either the age composition or the distribution of the escapement.

Available historical records were examined for evidence of these types of changes but largely with a negative result; therefore, the hypothesis was advanced that the observed declining rate of return per spawner is caused by a declining basic productivity of the nursery areas. The latter is then ascribable to the cumulative effect of relatively little enrichment of bioenergetic elements from salmon carcasses since the instigation of commercial fishing operations in comparison with the prefishing era when the entire virgin run escaped to the spawning grounds.

Suggestions are made for future field testing of this hypothesis.

In the development of the salmon fishery along the eastern perimeter of the Pacific Ocean, the most southern stocks were utilized first. As demand increased and certain stocks declined, the fishery shifted northward until the runs of the entire southeastern Alaska and soon thereafter those of the western districts were exploited. The rapidity of growth of the salmon fishing industry in Alaska is astonishing. The first cannery was built in southeastern Alaska at Klawak in 1878 (Rich and Ball, 1928), and only 6 years later exploratory fishing was conducted in Bristol Bay.

The early Bristol Bay catch records show that, from 1884 to 1891, fishing was conducted only in Nushagak Bay (Figure 1). Four years later, salmon was harvested in the other watersheds of Bristol Bay, the Kvichak-Naknek, the Egegik, and the Ugashik Districts. The patterns were initially alike, with a continuous and steady rise in production for at least 10 years in the smaller districts of Egegik and Ugashik and 20 years or more in the Nushagak District and even longer in the Kvichak District where on the average more than 60% of the Bristol Bay harvest is made annually.

As these four fisheries developed, annual variations became more and more apparent, but the overall production was fairly stable until 1919, when it declined drastically all over Bristol Bay in spite of no decline in fishing effort. The catches in Ugashik, Egegik, and Kvichak Districts soon thereafter rebounded to their former production level, but the catches in the Nushagak District did not. From this point on, the pattern of development in Nushagak differed from that of the other fishing areas in Bristol Bay, primarily in a more severe and persistent decline of the stocks making up the entire run.

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In an effort to reverse this downward trend by providing for larger escapements, fishing effort was reduced by restrictions on fishing time, gear, and location. The effect of these measures can be gauged from three principal sources of information: (1) A counting weir was operated in the Wood River of the Nushagak District, the principal trunk stream, during the years 1908-1919. (2) Biological studies were conducted in subsequent years that provided data on the age, length, and size composition of the catch and in part of the escapement. (3) The salmon canning industry itself has kept meticulous records on daily catches, number of fishing units, and type of gear operated.

The various sources of data indicated above were utilized to reconstruct the levels of escapements in the Nushagak District during the last 50 years in an effort to determine whether the magnitude of the yearly escapements is correlated with the declining salmon production in Nushagak Bay. If this were not the case, the fishery may have changed the age and size composition of the stock or the distribution of the various stocks in time and space. These factors will be examined in a search for a logical explanation of the decline of the Nushagak fishery.

NUSHAGAK BAY AND WATERSHED

Nushagak Bay includes the waters between a line drawn from Nichol's Spit to Etolin Point and the confluence of the Wood and Nushagak Rivers (Figure 1). These streams serve as the trunk streams of the Wood River lakes and the Tikchik lakes, respectively. Two other trunk streams drain into Nushagak Bay, namely, the Snake River and the Igushik River. The entire watershed comprises a drainage basin of 10,207 km^2. The morphometric parameters of some of the more important salmon-producing lakes are given by Gadau (1966).

Although sockeye salmon occur in more northerly latitudes, the Nushagak River system represents the northern boundaries of large sockeye salmon runs. The reason may be the absence of large lakes in more northern stream systems, which would provide sufficient nursery grounds. Thus, in the Tikchik system there are six lakes with five accessible to the salmon, but only the three lower ones, indicated on Figure 1, are important for sockeye salmon production.

NUSHAGAK SOCKEYE CATCHES, 1884-1966

The commercial fishery for sockeye salmon in Bristol Bay began in Nushagak Bay in 1884 after the schooner *Neptune* made an exploratory salting expedition (Moser, 1902). Prior to that time, some salting, from 800 to 1200 barrels each year, was done by fishermen operating a simple trap in the Wood River.

The most recent account of catch data was published by Kasahara (1963). His figures differ in some years from those given in Tables 1 and 2 of this paper, compiled in part from original sources, but the discrepancies are mostly minor in nature, and they do not change the overall picture in catch level and trend. Derivation of the Nushagak catch figures used in this report is given in the footnotes and comments to the mentioned tables.

When the Nushagak catches are plotted, they exhibit strong annual variations, as in most sockeye salmon runs (Figure 2). A small part of the variability can be explained by differences in fishing effort, which reflected economic conditions or inaccurate predictions by the cannery superintendents as to the actual size of the run. Viewed over longer time periods, however, there can be no doubt that the annual catches reflect changes in stock strength. This conclusion is amply brought out by the construction of a trend line by a moving average of 5's because of 5-year cycle.

Three distinct periods are discernible. The first period spans the years 1900-1918, the second one covers the years 1921-1945, and the last period includes the years 1946-1966. The average annual catches during these periods were 5,134,156; 2,888,726; and 1,183,485 salmon, respectively.[3]

Transition from one level to the next took

[3] If the estimated foreign catches made since 1956 were included with the domestic catches for the third period, the average annual catch would be raised about 25%.

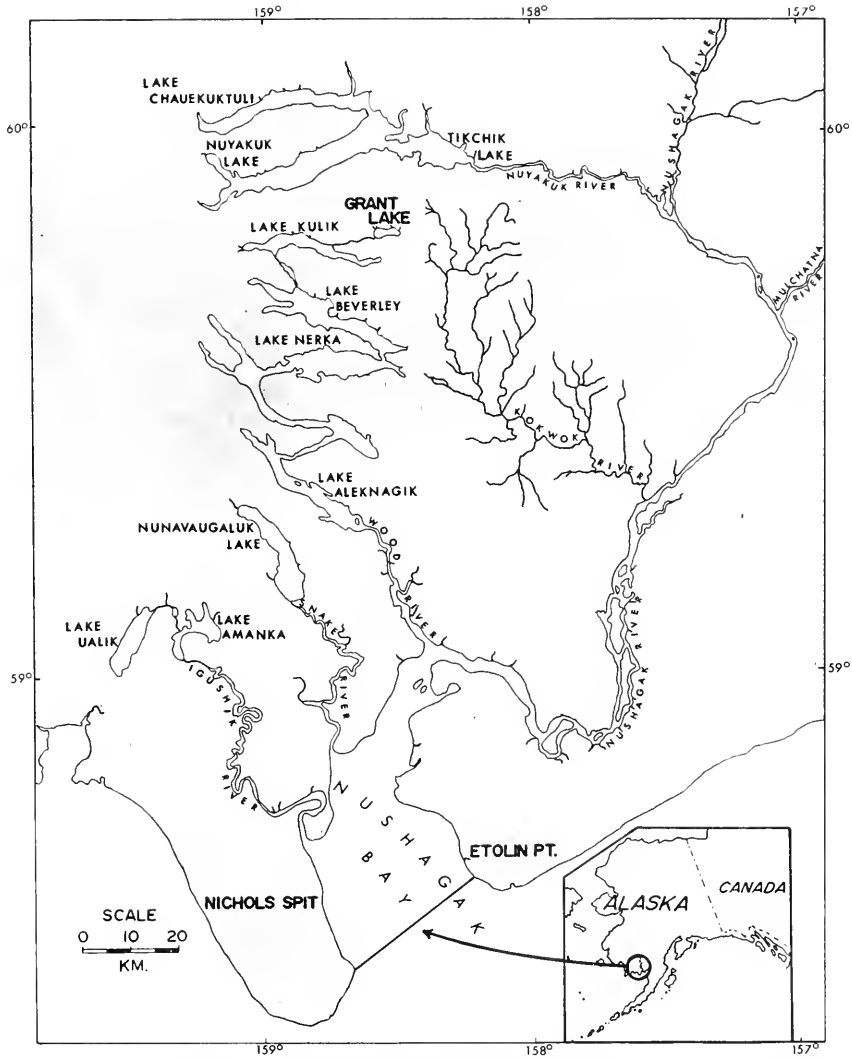

FIGURE 1.—The Nushagak District of Alaska showing (from north to south) the Tikchik, Wood River, Snake River, and Igushik River lake systems.

TABLE 1.—Commercial catches of sockeye salmon, Nushagak Bay, 1893-1945.

Year	Number of fish	Year	Number of fish
1893	640,000	1921	3,717,284
1894	860,000	1922	3,408,358
1895	938,946	1923	1,921,874
		1924	2,168,154
1896	1,262,690	1925	3,903,125
1897	1,240,080		
1898	1,890,092	1926	4,022,328
1899	2,517,436	1927	657,467
1900	4,234,533	1928	4,957,096
		1929	3,851,479
1901	5,401,051	1930	1,610,568
1902	4,725,715		
1903	6,319,189	1931	2,260,541
1904	5,345,659	1932	3,083,615
1905	7,387,935	1933	3,753,230
		1934	4,575,049
1906	5,427,512	1935	649,093
1907	2,627,351		
1908	6,092,031	1936	1,560,138
1909	4,906,635	1937	4,561,298
1910	4,469,755	1938	2,322,704
		1939	4,169,121
1911	2,957,073	1940	1,519,082
1912	3,993,428		
1913	5,409,933	1941	1,897,869
1914	6,457,815	1942	2,465,779
1915	5,904,862	1943	3,373,643
		1944	3,513,241
1916	3,744,551	1945	2,296,019
1917	5,847,239		
1918	6,296,702		
1919	1,477,336		
1920	2,682,056		

Sources:
1884-1927 — Rich and Ball (1928)
1929-1945 — Annual District Management Reports, District Agents Bureau of Fisheries and Fish and Wildlife Service.

Comments:
The catches for 1884-1892 are only given in cases and therefore are not included.
For the years 1925-1946, Alaska Salmon Industry gathered data on the catch, the pack, and expended effort by the major fishing companies.
The number of fish per case is computed from the information collected by Alaska Salmon Industry. It was used for conversion of the case pack into number of fish for the years 1929, 1930, 1931, 1932, and 1941, since only the case pack is recorded for these years in the Reports of the Management Agencies.
One 200-lb barrel of salted salmon has been set equal to 54 fish and one 350-lb barrel equal to 95 salmon.
The official records for the year 1928 list only canneries that operated in Nushagak in this year. The catch figure used is based on records submitted to Ala-ka Salmon Industry from all but two canneries. The catch in the latter case was extracted from the sworn reports submitted by the fishing industry to the tax authorities.

place within 2 to 3 years. Although the other districts in the Bristol Bay region have experienced a decline in production, this decline has been neither so distinct nor so drastic in nature as in the Nushagak District.

FISHING GEAR AND AREAS IN NUSHAGAK BAY

Three types of fishing gear have been utilized in Nushagak Bay: traps, drift gill nets, and

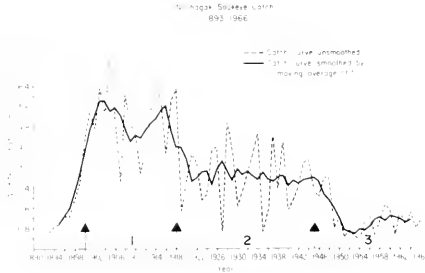

FIGURE 2.—Catches of sockeye salmon in the Nushagak fishery, 1893-1966.

stationary gill nets (set nets). Traps were not used to any great extent in the Nushagak fishery or in Bristol Bay as compared with other areas of Alaska, in which they were in widespread use. The main factor which discouraged the use of traps undoubtedly was the strong tidal currents in Bristol Bay, where tidal differences reach as high as 25 ft or more and peak water velocities reach 4 to 5 knots. These conditions permitted trap operations only in a restricted number of places. Since gear records became available in 1904 and until traps were outlawed in 1923, their number in Nushagak Bay varied from 3 to 11 (Rich and Ball, 1928).

Apparently set nets were not commonly used during the period when traps were legal. The first documented set net catches were taken in 1924, and set nets are mentioned in the 1926 regulations. A maximum length of 75 fm was set in 1926, but in 1931 maximum length was reduced to 50 fm, as is the case today. Since the advent of yearly reports by the management agent in 1929, accurate records have existed as to the distribution of effort between these two types of gear.

Up to and including 1922, no restrictions were placed on mesh size and length of the drift gill nets. In 1924, the maximum length of drift nets was set at 200 fm and mesh size of at least 5¾ inches, stretched measure, between knots. After the 1925 season, minimum size was set at 5½ inches. No other changes in mesh regulations

TABLE 2.—Catches and escapements of sockeye salmon in Nushagak District, 1946-1966.

Year	Catch	Escapement by river system			Estimated total run	Escapement as percent of total run
		Wood River	Other streams	Total		
1946	2,028,144	3,717,000	1,002,000	4,719,000	6,747,144	70 0
1947	2,767,287	1,782,000	725,000	2,507,000	5,274,287	47.5
1948	2,805,793	1,483,250	608,000	2,091,250	4,897,043	42 7
1949	800,123	101,025	37,000	138,025	938,148	14 7
1950	1,212,091	451,600	121,000	572,600	1,784,691	32 1
1951	436,950	457,600	82,000	539,600	976,550	55 3
1952	698,071	226,800	207,000	433,800	1,131,871	38 3
1953	449,341	515,542	313,000	828,542	1,277,883	64.8
1954	315,357	570,624	121,000	691,624	1,006,981	68.7
1955	1,054,978	1,382,755	551,000	1,933,755	2,988,733	64 7
1956	1,263,186	773,101	439,000	1,212,101	2,475,287	49.0
1957	491,498	288,727	210,000	498,727	990,225	50 4
1958	1,092,156	960,455	317,478	1,277,933	2,370,089	53.9
1959	1,719,687	2,209,266	832,619	3,041,885	4,761,572	63.9
1960	1,517,988	1,016,073	657,185	1,673,258	3,191,246	52 4
1961	511,483	460,737	398,896	859,633	1,371,116	62.7
1962	1,461,766	873,888	63,810	937,698	2,399,464	39 1
1963	842,744	721,404	342,452	1,063,856	1,906,600	55.8
1964	1,420,941	1,076,112	262,892	1,339,004	2,759,945	48 5
1965	793,323	675,156	424,110	1,099,266	1,892,589	58.1
1966	1,170,271	1,208,682	422,044	1,630,726	2,800,997	58 2
Total	24,853,178	20,951,797	8,137,486	29,089,283	53,942,421	--
Average 1946-1966	1,183,485	997,705	387,499	1,385,204	2,568,689	51.9

Sources
1946-1959 — Mathisen, Burgner, and Kao (1963)
1960-1966 — Alaska Department of Fish and Game, Division of Commercial Fisheries, Bristol Bay Area, Annual Management Report 1966, 59 p.

were made until 1961, when 5¾-inch nets were permitted.

The length of drift nets was reduced to 150 fm per boat in 1929 and has remained unchanged. However, the industry did not necessarily always feel compelled to observe these maximum and minimum limits. The gill net fishery developed before such regulations were introduced and enforced. The necessity of observing a correspondence between the pull of the boat and the drag of the gill net had more or less standardized the gear. Length of the drift net and mesh size as actually used can be studied from two other sources (Table 3).

For the period 1902-1925, the mesh sizes and lengths of the drift nets used were given in the yearly reports of two canneries belonging to Alaska Packers Association in Nushagak.

Since 1926 reports have been submitted by the operators to the Federal government concerning their canning activities. These sworn statements give the lengths of nets used by the different companies. Since the advent of statehood, gear regulations have been published annually.

There has been a gradual but steady decline of the mesh size to 5¾ inches, stretched mesh. In contrast, the length of the drift nets has been remarkably constant. Even when an upper limit of 200 fm was introduced in 1924, many operators used nets half this size or 100 fm. Since 1928, all drift nets have measured 150 fm long.

TABLE 3.—Mesh sizes and lengths of drift gill nets used in the Nushagak sockeye salmon fishery, 1902-1966.[a]

Year	Stretched mesh size	Length of drift net
	inches	*fathoms*
1902	6⅛-6¼	--
1903	6⅛	120
1904	6-6⅛	150
1905	6⅛	150
1906	6-6⅛	150
1907	6⅛-6¼	160
1908-1912	6	160
1913-1925	5¾	150[b]
1926-1927	5½-5¾	100-200[c]
1928-1960	5½	150[d, e]
1961-1966	5⅜	150

[a] Information prior to 1925 taken from records of the Alaska Packers Association and for subsequent years from announced regulations of the Federal Authorities.
[b] A maximum legal length of 200 fm and minimum mesh size of 5¾ inches specified for 1924 and 1925.
[c] A minimum mesh size of 5½ inches set for new nets.
[d] A maximum length 150 fm set in 1929.
[e] A maximum length of 100 fm set for 1937 only.

except for the year 1937, when the maximum size was reduced to 100 fm for 1 year and only for Nushagak Bay.

Powered fishing boats were outlawed in 1922 and not permitted again until 1951. However, in the 30's the canning companies started to use small tug boats to tow the fish boats from one place to another, or most commonly to assist in bringing a boat to the delivery scow. Consequently, the efficiency of one boat increased with this added mobility. In part, it was offset by the movement of fishing boundaries over the years farther and farther out from the river mouth and thereby reduction in efficiency of the fishing gear.

In 1899, fishing above tidewater was prohibited in streams less than 500 ft in width. In the tidewater of smaller streams, gear could only cover one-third of the stream width.

In 1907, fishing in the Wood and Nushagak Rivers was prohibited within 500 yards of the mouth of Wood River. Over the years, gradually, restrictions of fishing area have been imposed, which resulted in a transfer of fishing operations away from the river and river mouth and into the open Nushagak Bay. In Figure 3 are indicated locations of canneries in operation shortly after the turn of the century. Only three plants remain actively canning in Nushagak Bay today.

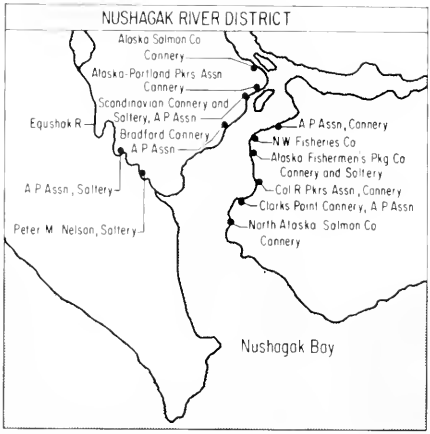

FIGURE 3.—Copy of an old map (probable date 1907) with the locations of canneries and salteries in Nushagak Bay. Canneries in operation today are the Columbia-Wards Cannery at the site of North Alaska Salmon Company Cannery, a Queen Fisheries plant near Columbia River Packers Association plant, and Pacific Alaska Fisheries Dillingham plant at the site of Alaska-Portland Packers Association.

THE NATURE OF THE SOCKEYE SALMON RUNS

All sockeye salmon runs to Bristol Bay have a very distinct and regular time schedule. Historically, the period from June 25 to July 25 has been considered as the time when the salmon are present in Nushagak Bay in catchable quantities. Records accumulated since 1955 indicate that, on the average, peak catches in Nushagak Bay were made on July 5 (Royce, 1965).

The entry is of a pulse type with exponential declining departure curves for the trunk streams and the spawning grounds (Mathisen, 1969). Bi- or trimodal catch curves, especially in earlier years, undoubtedly were created by changes in frequency or relative strength of the individual pulses. We do not know the racial composition of the individual pulses, but some tagging data (Straty, 1969) point to a fairly random mixing of individual races.

Basically, the juvenile salmon spend 1 or 2 years after emergence in the nursery areas of the freshwater lakes. They return from ocean feeding after 2 or 3 years. Thus, four different age groups will make up a year's run, namely, 1.2, 1.3, 2.2, and 2.3 (after Koo's [1962] notation). The number of fish in other age groups is insignificant and can be disregarded. The Nushagak District differs from other districts in Bristol Bay in having a preponderance of 1-freshwater check salmon.

ESCAPEMENT LEVELS IN THE NUSHAGAK DISTRICT

The history of the Nushagak fishery was divided earlier into three periods. Within each of these periods data exist in regard to escape-

ment levels, although they differ in completeness. Naturally the best records have been assembled during the last period, while the most incomplete records exist from the middle period. The escapement records are discussed in order of completeness.

THE PERIOD 1946-1966

The records for this period are complete in the sense that escapement estimates were made for all streams draining into Nushagak Bay (Table 2). Estimates were based originally on ground or aerial surveys. In 1953, the Wood River escapement was estimated from tower counts in the trunk stream. In 1958, this technique was adopted for assessment of the Igushik escapement, and in 1959 for that of the Nuyakuk River. From 1960 to 1964, tower counting was conducted in the Snake River system and inaugurated in the Nushagak-Mulchatna River in 1966. Otherwise, escapement estimates were made by the less reliable aerial survey. The earlier estimates based on ground surveys all have in common a much larger variance, but they gain in consistency because of the fact that largely the same personnel conducted spawning surveys in all years, even after introduction of tower counts (Gilbert, 1968).

In the present context, we are not primarily interested in the year-by-year changes in catch and escapement level, rather in the overall ratio of catch to escapement over the entire period. The average catch amounted to 1,183,485 sockeye salmon and the average escapement to 1,385,204 spawners. The ratio is almost one to one; or on the average, a pair of spawners produced a progeny of four fish. In terms of fishing mortality, the rate of exploitation has averaged 48.1%.

THE PERIOD 1908-1919

No total escapement estimates exist for this period, except for the Wood River system where a counting weir was operated from 1908 to 1919 with the exception of the 1914 season. Daily counts and comments on weir building and maintenance are found in Reports of the Commissioner of Fisheries for the years in question (Table 4).

On the assumption that the ratio of the Wood River escapement to the total Nushagak escapement was the same for the years 1908-1919 as was observed during the period 1946-1966, when estimates were available of the Wood River escapement as well as of the total Nushagak escapement, then this ratio can be used to estimate the total Nushagak escapement for the years 1908-1919 from the weir counts.

The ratio of the total Nushagak escapement to the Wood River escapement has been computed in two ways from data in Table 2. The first ratio

TABLE 4.—Total Nushagak escapement 1908-1919.

Year	Wood River weir count	Estimated Nushagak escapement	Catch	Estimated total run	Escapement as percent of total run
1908	2,603,655	3,758,636	6,092,031	9,850,667	38.2
1909	893,244	1,289,487	4,906,635	6,196,122	20.8
1910	670,104	967,362	4,469,755	5,437,117	17.8
1911	354,299	511,466	2,957,073	3,468,539	14.7
1912	325,264	469,551	3,993,428	4,462,979	10.5
1913	753,109	1,087,188	5,409,933	6,497,121	16.7
1914	No count	--	6,457,815	--	--
1915	259,341	374,385	5,904,862	6,279,247	6.0
1916	551,959	796,808	3,744,551	4,541,359	17.5
1917	1,081,508	1,561,265	5,847,239	7,408,504	21.1
1918	943,202	1,361,606	6,296,702	7,658,308	17.8
1919	145,114	209,487	1,477,336	1,686,823	12.4
Total	8,580,799	12,387,241	57,557,360	63,486,786	--
Average 1908-1919	780,073	1,126,113	4,796,447	5,771,526	19.5

Sources: Reports of the Commissioner of Fisheries for the fiscal years 1908 through 1919 and special papers. Government Printing Office, Washington, D.C., 1910-1921.

is based on the data for the years 1946-1957, when the escapement estimates were made largely from ground and aerial surveys. Presumably this estimate is less reliable than the second estimate based on the years 1958-1966, when the escapement estimates were based largely on very reliable tower counts. The two ratios are 1.4438 and 1.4433.

Thus the average Wood River escapement has formed a remarkably constant proportion of the total Nushagak escapement regardless of which years are used for calculation. Consequently, the ratio 1.4436, based on data for all the years 1946-1966, has been used to enlarge the early Wood River weir counts from 1908 to 1919 to reflect the Nushagak escapement for the same years.

Estimated in this manner, the annual Nushagak escapement for the period 1908-1919 averaged 1,126,113 spawners, and the total Nushagak sockeye run averaged 5,771,526 salmon, or a rate of exploitation of 80.5%.

THE PERIOD 1925-1945

No weir counts exist for these years, and quantitative stream surveys were not conducted. However, escapement estimates can be made from available data on the catch, size distribution of the fish, sex ratio, and expended effort.

From 1926 on, the legal minimum mesh size was 5½ inches, and from 1927 the maximum length of the drift gill nets remained unaltered at 150 fm. An exception must be made for 1937, when the maximum length was reduced to 100 fm for this one year in the Nushagak fishery. Therefore, given a measure of the catch and the instantaneous rate of fishing for each centimeter group by the 5½-inch gill nets and an estimate of expended fishing effort, the escapement can be calculated by centimeter groups from the formula for competitive fishing units and summed over the size range observed in a year to give the total escapement:

$$E = \sum_{j=a}^{b} C_j \frac{1}{e^{fq_j} - 1},$$

where E = the unknown total escapement,
C_j = the known catch for size group j,
q_j = the coefficient of catchability for size group j,
f = the number of standardized fishing units, and
a and b = lower and upper bounds of the size range.

No natural mortality has been assumed during the fishing season.

Because of the different selection curves for males and females by 5½-inch mesh size, these calculations must be done separately for each sex. The necessary data for this calculation follow.

Sex Ratios

It has been assumed that no selection for sex was exerted in the collection of samples for size and age composition. Consequently, the numbers of males and females measured in a day provide an estimate of the sex ratio in the catch for that particular day. This procedure was necessitated by the absence of specific sex ratio samples.

Size Composition of the Catch

During the years considered here, the Bureau of Fisheries stationed biologists at selected canneries for collection of scale samples and length measurements. At other times, resident people were hired for the same purpose and paid a fixed amount for each scale book collected.

Generally the type of length measurements made is not indicated in the records; but it has been assumed that the procedure was to measure length from the tip of the snout to the fork of the tail. This assumption was verified by a comparison of the resulting length-frequency curves with the mean lengths of 2- and 3-ocean fish in postwar years.

Since 1946, the common procedure has been to measure the length of the sockeye salmon taken in the fishery from the middle of the eye to the fork of the tail. The Fisheries Research Institute took a series of double measurements in 1946 to provide a basis for constructing a regression line between the two types of measurements, and a conversion can be made from one

measurement to the other by means of the two following equations:

$$\delta : \text{ME-TF} = 536.772 + 0.8279\,[(\text{snout-TF}) - 592.340]$$

$$\varphi : \text{ME-TF} = 527.481 + 0.8946\,[(\text{snout-TF}) - 569.724]$$

Commonly, length measurements were collected throughout the fishing season. These measurements were grouped by fishing periods or by time periods for which catch records exist. Finally, a seasonal weighted length-frequency distribution was computed by the use of the period catches as weighting factors.

Expended Fishing Effort

Batts and Fischler (1967) have summarized the fishing regulations promulgated during the years 1924-1945. A summary of the allowable fishing time is given in Table 5, without consideration for the stage of the tide in relation to closed and open periods. Although the largest or smallest tides generally are inferior fishing periods compared with the medium-sized ones, no correction was attempted on the premise that the plus and minus deviations tended to cancel each other over the entire season.

The number of fishing boats that operated each year for the period 1929-1945 is recorded by the management agents in their annual reports and copied in Table 5. The size of the Nushagak fishing fleet in 1925-1928 was estimated from the data collected by the Alaska Salmon Industry. More than 60% of the total Nushagak catches during these 4 years were made by the reporting canneries, which also submitted records on the number of boats employed. By direct proportionality an estimate was derived for the total number of fishing boats and set nets that operated from 1925 to 1929 (Table 5).

So that a common unit of effort could be derived, the fishing power of set nets was expressed in terms of that for drift nets according to a method by Robson (1961).[4] The conversion was made separately for each year by consideration

[4] Robson, D. S. 1961. Estimation of the relative fishing power of individual ships. Cornell Univ., Biometrics Unit, Plant Breeding Dep., BU-133-M. (Unpublished manuscript.)

TABLE 5.—Registered fishing effort in Nushagak Bay, Bristol Bay, Alaska, 1925-1950.

Year	Total fishing time in days	Total number boats (drift nets)	Total boat day units	Total number set nets	Total set net day units	Relative efficiency set nets/boats	Set net units converted to boat units	Total effort in boat days
1925	22.000	337	7,414	66	1,452	.0717	104.0	7,518
1926	21.000	256	5,376	44	924	.1153	106.5	5,483
1927	19.500	292	5,694	68	1,326	.2796	370.7	6,065
1928	22.500	264	5,940	39	878	.3283	288.7	6,229
1929	21.000	311	6,531	115	2,415	.3613	872.5	7,404
1930	15.250	335	5,109	112	1,708	.1640	280.1	5,389
1931	13.500	351	4,739	152	2,052	.3264	669.8	5,409
1932	18.333	276	5,060	208	3,813	.1151	438.9	5,499
1933	18.688	280	5,233	167	3,121	.1617	504.7	5,738
1934	19.666	279	5,487	221	4,346	.0833	362.0	5,849
1935	9.000	65	585	154	1,386	.2981	413.2	998
1936	17.750	298	5,290	263	4,668	.5129	2,394.2	7,684
1937	19.500	236	4,602	173	3,374	.1594	537.8	5,140
1938	19.500	99	1,931	96	1,872	.2426	454.1	2,385
1939	19.000	235	4,465	144	2,736	.3217	880.2	5,345
1940	14.000	129	1,806	128	1,792	.5773	1,034.5	2,841
1941	19.000	125	2,375	116	2,204	.1803	397.4	2,772
1942	27.500	96	2,640	53	1,458	.2827	412.2	3,052
1943	21.500	119	2,559	98	2,107	.3850	811.2	3,370
1944	24.000	118	2,832	103	2,472	.2889	714.2	3,546
1945	20.000	82	1,640	164	3,280	.2134	700.0	2,340
1946	16.500	198	3,267	119	1,964	.3077	604.3	3,871
1947	21.000	181	3,801	190	3,990	.2650	1,057.4	4,858
1948	16.000	198	3,168	216	3,456	.4709	1,627.4	4,795
1949	10.400	192	1,997	272	2,829	.2255	637.9	2,635
1950	13.500	108	1,458	270	3,645	.1728	629.9	2,088

of 5 days during the peak of the fishing season. This procedure eliminated some of the variability present at the beginning or the end of the fishing season due to irregular entries or departures of the salmon. The choice of 5 days was made in order to avoid too complicated a scheme, and often more than half of the total Nushagak catch was taken during the time period considered.

In this two-way classification with two rows corresponding to drift net and set net and five columns corresponding to the time periods, the catch in 1 day and by a given type of gear is:

$$C_{ij} = f_{ij} \cdot r_i \cdot N_j \cdot \epsilon_{ij} ,$$

where f_{ij} = the number of fishing units of type i operated on day j,
r_i = the coefficient of catchability of gear type i for all size groups,
N_j = the average stock of salmon encountered by the gear on day j, and
ϵ_{ij} = error term.

If $r_{\cdot\cdot}$ is the coefficient of catchability of a unit of a theoretical average of all types of gear, one can write $\alpha_i = r_i/r_{\cdot\cdot}$. Similarly, if the average stock size encountered by the gear during the entire period is defined as $N_{\cdot\cdot}$, one has $\beta_j = N_j/N_{\cdot\cdot}$. Finally, the error term was considered log-normal (Beverton and Holt, 1957).

The random variable $Y_{ij} = \log (C_{ij}/f_{ij})$ can be written then as

$$Y_{ij} = m + a_i + b_j + e_{ij},$$

Since we have only two types of gear, $\log r_2 - \log r_1 = a_2 - a_1$. An estimate of a_1 can be obtained directly from a linear hypothesis program, such as BMD 05V (Dixon, 1965), under the constraint $a_1 + a_2 = 0$. The results expressed as arithmetic ratios are listed in Table 5.

Fishing Power of 5½-Inch Gill Nets

Two size groups of fish predominate in all Bristol Bay sockeye salmon fisheries (Mathisen, Burgner, and Koo, 1963). The 3-ocean fish measure on the average from 5 to 6 cm longer than the 2-ocean fish, and the males of both size groups are between 2 and 3 cm larger than the females.

Between years there are pronounced differences in the proportion of 2- and 3-ocean fish and, to a much smaller extent, in the sex ratio of the total runs. Since during the middle part of the Nushagak fishery considered here, the mesh size of the gill nets remained stable at 5½ inches, the total fishing mortality generated by one unit of gear changed from year to year primarily with changes in the relative proportion of 2- and 3-ocean fish and males and females. Consequently, the coefficient of catchability must be determined by length or age groups, and separately for males and females. There are only 5 years, 1946-1950, with records of catch and escapement when sailboats were used together with linen gill nets. Conversion to powered fishing boats was largely accomplished by 1954, although a shift in boat types continued. At the same time nylon gill nets came into universal use. Added to these changes were modifications of boundary lines of the fishing districts. Therefore, the rate of the present-day fishing of the gear in Bristol Bay is not comparable with that which prevailed during the middle period of the Nushagak fishery.

Data on catch and escapement and the corresponding length-frequency distributions for Nushagak from 1946-1950 are available (Mathisen et al., 1963). The escapements were estimated visually and may not be too accurate. But in 1 year, 1946, when an independent estimate could be made from a tagging experiment, the correspondence was remarkably great (Mathisen, 1969). Effort during the same years is listed in Table 5. On the assumption that set net effort can be converted into drift net effort and that all units of gear were fishing simultaneously on the same stock, it is a straightforward matter of computing the coefficient of catchability for each centimeter group and separately for males and females from the expression on page 754 used in reverse.

There were rather large year-to-year variations; therefore the following smoothing process has been applied to the data. An arithmetic mean value for each centimeter group was found for the 5 years considered. A moving average of 5's of these arithmetic means provided the final values in the selection curves in Figure 4. The dip in the selection curve for males is con-

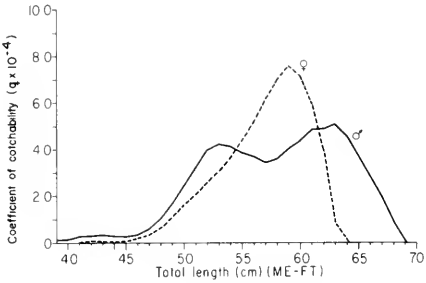

FIGURE 4.—Instantaneous rate of fishing by centimeter groups.

sidered due to statistical variability introduced by the rather small escapements in 1949 and 1950. It was further demonstrated by similar calculations for recent years with exact catch and escapement data that once the males become vulnerable to the gear, the coefficient of catchability increases only slightly from 2- to 3-ocean fish. Whereas in the lower part of the selection range, the curves are fairly similar for males and females, the rate of fishing on 3-ocean females was several times that of 2-ocean females based on the average lengths of these two groups given by Mathisen et al. (1963). As a result, in years when 2-ocean fish predominated in the run, a large preponderance of females was present in the escapement as in 1946, when there were 68% males in the catch and only 35% males in the escapement. In 1948, when there was a predominance of 3-ocean fish, the corresponding figures were 44% and 49%.

The selective action of the gill nets on the 3-ocean fish can be demonstrated further by comparison of age composition of gill net and trap catches made in the same year (Table 6). The traps can be considered nonselective and were placed close to the upper boundary line of the fishing area (Moser, 1902). Therefore, the age composition of the trap catches can be used as an estimate of the age composition of the escapement. Whereas the 2- and 3-ocean fish were present in about the same numbers, the catch by 5¾-inch gill nets contained more than five times as many 3-ocean fish as 2-ocean fish.

TABLE 6.—Age composition in trap and gill net catches in Nushagak, July 1 and 5, 1919.

Age group	Traps		Gill nets	
	Number of fish	Percent	Number of fish	Percent
1.2	65		14	
2.2	33		15	
2-ocean	98	51.9	29	15.5
1.3	83		150	
2.3	8		8	
3-ocean	91	48.1	158	84.5
Total	189		187	

Source: Clark, Frances N. 1933. Red salmon in the Nushagak District of Bristol Bay, Alaska. (U.S. Bureau of Fisheries) Natl. Mar. Fish. Serv., Biol. Lab., Auke Bay, Alaska. (Unpublished manuscript.)

ESTIMATED ESCAPEMENTS, 1925-1945

When the calculations outlined above are executed, an estimated escapement for each of the years from 1925 to 1945 is obtained (Table 7). Two years, 1932 and 1938, were not included in the computation of an average escapement level since no length measurements were taken in these years. No measurements were taken in the fishery in 1931; instead, scales and measurements were collected in the Wood River, and this length-frequency distribution has been

TABLE 7.—Calculated escapements and total runs in Nushagak District, 1925-1945.

Year	Total catch	Total escapement	Estimated total run	Escapement as percent of total run
1925	3,903,120	285,081	4,188,201	6.8
1926	4,022,333	697,730	4,720,063	14.8
1927	657,468	803,643	1,461,111	55.0
1928	4,957,072	1,383,130	6,340,202	21.8
1929	3,851,482	754,125	4,605,607	16.4
1930	1,610,568	3,158,751	4,769,319	66.2
1931	2,260,539	491,877	2,752,416	17.9
1932	3,083,165	--	--	--
1933	3,753,230	1,995,688	5,748,918	34.7
1934	4,575,043	1,791,481	6,366,524	28.1
1935	649,093	2,277,858	2,926,951	77.8
1936	1,560,135	1,816,382	3,376,517	53.8
1937	4,561,297	10,118,033	14,679,330	68.9
1938	2,322,704	--	--	--
1939	4,169,122	361,356	4,530,478	8.0
1940	1,519,082	990,237	2,509,319	39.5
1941	1,897,870	1,197,981	3,095,851	38.7
1942	2,465,779	1,586,861	4,052,640	39.2
1943	3,373,650	1,762,232	5,135,882	34.3
1944	3,513,236	1,335,734	4,848,970	27.6
1945	2,296,020	1,614,470	3,910,490	41.3
Total	61,002,458	34,422,650	90,018,789	--
Average 1925-1945	2,904,879	1,811,718	4,737,831	38.2

used. Since the fish in the escapement average smaller than in the catch, it will result in an overestimate of the total run for this year.

Unquestionably, the computed escapements are subject to many sources of error, and they reflect only the general magnitude of the escapements. In general there are some measurements from each fishing period that can be weighted by the corresponding catches, and any unrepresentativeness of the sampling was in part corrected. It therefore appears that the greatest bias arises from the way in which fish were selected and measured. In 1930, for example, there were few measurements taken, and they included a rather high proportion of suspiciously small 2-ocean females, which resulted in the rather large estimated total escapement. Almost 12,000 measurements were made in 1937, but largely of fish from the resident set net fishery near the upper fishing boundary. The result is an underestimate of the mean average length in the commercial catches, since the run at this point had been subjected to the selection of the drift net fishery; the calculated escapement is substantially inflated. In 1939 no 2-ocean fish were measured in drift net catches, and therefore the low rate of escapement may be substantially correct.

The International North Pacific Fisheries Commission (1962) has published estimates of Nushagak escapements for the period considered here. A fishing rate common for all size groups, and with no distinction between males and females, was computed from Bristol Bay catch and escapement data for 1955-1957. Furthermore, nylon gill nets were used and were operated from power boats. Because of the selective action of the gill nets for males and females, and for 2- and 3-ocean fish, it is easy to understand that these estimates are entirely different from those presented here.

SUMMARY OF RESULTS

On the previous pages, escapement levels were calculated for the three distinct periods of the Nushagak fishery shown by the catches on Figure 2. These results have been summarized in Table 8.

TABLE 8.—Rate of exploitation in three periods of the Nushagak fishery.

Period	Average escapement	Average catch	Exploitation
	Thousands	*Thousands*	*%*
1908-1919	1,126	4,796	81
1925-1945	1,812	2,905	62
1946-1966	1,385	1,183	48

During the early period of the fishery, the runs sustained a fishing mortality of more than 80% until 1919 when all runs to Bristol Bay suffered a drastic decline. The universality of this decline in many sockeye salmon systems suggests that the causes must be sought in changes in the environment and not in the mode of fishing operation. The Nushagak runs never returned to their former level, in contrast to those of the other systems in Bristol Bay, notably those to the Kvichak River.

During the middle period, here defined as the time from 1925 to 1945, the amplitude of the year-to-year oscillation increased (Figure 2).

Following the last World War, not only did the Nushagak and other Bristol Bay sockeye salmon runs decline, but many of the Kamchatka salmon runs did too (Krogius and Krokhin, 1956). The widespread decline suggests again that environmental and probably oceanographic conditions not related to fishing depressed the survival. In the third period of the Nushagak fishery the runs remained at a very low level, compared with levels of the two previous periods.

Concomitant with this stepwise decline in average yield, there has been a decrease of the reproductive potential of the Nushagak sockeye salmon runs. Whereas during the early period of the Nushagak fishery, the runs were exposed to an exploitation rate of nearly 80%, during the middle period of the Nushagak fishery, the runs were exposed to an exploitation rate of around 60%. During the last period, the exploitation rate was around 50%, largely set by the regulation. The runs are maintaining themselves, but so far no substantial increase is apparent.

Thus the rate of return per spawner has fallen from five to less than three and finally to two mature fish. As a result, there has been no increase to former run levels in spite of the reduced

exploitation rates. This situation is in contrast to the situation in the Fraser River, where the removal of the Hell's Gate blockade and increased escapements initiated almost an immediate increase in the returns in some river systems.

Therefore, it remains for us to explore if any changes have occurred in the Nushagak runs that can explain the described reduction in reproductive potential.

DISCUSSION

No visible changes have taken place in the Nushagak environment since fishing commenced there before the turn of the century. Even today there are no dams or any other obstruction to the migrating salmon. The resident population still remains so low that pollution problems or any form of industrial waste are nonexistent. Neither has the subsistence fishery increased in volume and an estimated 30,000 or more of all species are harvested today. Other freshwater fishes were not or were lightly harvested until recent years, when a recreational fishery for trout and char has developed.

A sockeye salmon run to a watershed such as the Nushagak District is made up of a great number of races that differ in morphological features, age structure, time and place of spawning, and reproductive rate. The most direct effect of overfishing would be the disappearance of certain races, or at least a reduction in their numerical size to the point where they cease to be important contributors to the commercial catches. If this were true, it could manifest itself on the spawning grounds after the various races have segregated. The number of spawners per unit of nursery area reflects the stock strength on a spatial basis.

There are some river systems within the Nushagak District with low spawning density relative to that of others. For 1955-1962 the average number of spawners per square kilometer of lake rearing area in the Tikchik Lakes was 280 and in Lake Nunavaugaluk 290. In contrast, the spawning density in the Wood River lakes was 2,340 fish per square kilometer of lake rearing area and 4,360 fish in the Igushik system (Burgner et al., 1969). There is no evidence available to indicate that this was different in the early history of the Nushagak fishery. While there are relatively more 3-ocean fish in the Tikchik runs than elsewhere in the Nushagak system and hence a higher fishing mortality, the scarcity of spawning beaches and streams precludes both here and in the Nunavagaluk system the possibility of a large population prior to commercial exploitation.

The possibility still remains that the individual races may pass through the fishery at different times and thereby be exposed to different fishing rates. If this were so, one might expect to see some shift in time when peak abundance occurred. This was studied by plotting the dates when 10, 50, and 90% of the commercial catches were made (Figure 5). From 1895 to 1947, the two first points were reached at the same time aside from simultaneous year-to-year variations. There are some indications in Figure 5 that salmon were present longer in Nushagak Bay in years prior to 1920, but when one considers the exponential rate of departure to the spawning streams from the fishing grounds, the larger total runs during these years would account for such a prolongation of the fishing season. Added to this consideration is the fact that the canneries

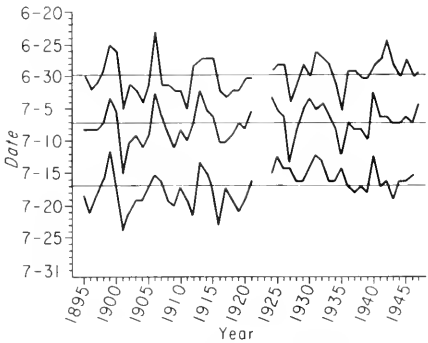

FIGURE 5.—Data on which 10, 50, and 90% of the Nushagak catch was made, 1895-1947. (Subsequent years omitted since a progressively stricter curtailment of fishing time prevented direct comparison with former years.)

usually set production goals in this period and extended fishing until these goals were reached.

As a result, one may conclude that 70 years of intensive harvesting have not drastically affected the timing of the Nushagak runs. All tagging experiments conducted in Bristol Bay point to a complete mixing of all races in the fishery and exposure to the same fishing pressure in a specific river system (Smith, 1964; Mathisen, 1969; Straty, 1969). Thus there is very little evidence of a differential rate of removal in time among all the races that constitute the Nushagak sockeye salmon run. The only exception seems to be the races bound for the Igushik system. Their migration path follows the west side of Nushagak Bay past Nichol's Spit. In earlier years when the main fishing activities were concentrated closer to the confluence of the Nushagak and Wood Rivers than they are today, the fishing pressure on the Igushik races during those years was lower.

A differential fishing pressure could arise from the selectivity of the gill nets if some Nushagak races consisted primarily of 3-ocean fish while in others 2-ocean fish predominated. Burgner (1964) has pointed out the preponderance of 3-ocean fish to the Tikchik as one example. However, if a diminution of such races were of any real consequence, it must manifest itself in changes of the age composition through the recorded history of the Nushagak fishery. The figures in Table 9 are based on the age composition in the commercial catches. Because of the larger net sizes used up to 1926, a bias is introduced in favor of 3-ocean fish and only the last two periods are directly comparable.

Throughout all years the majority of the fish migrated to sea as age 1 smolts and returned in somewhat the same proportion of 2- and 3-ocean fish. Over the years one can notice a shift, with less 3-ocean fish in the catches of males in recent years. If similar data were available for the escapement, and thereby of the total runs, one would in all probability see more of a contrast in the shift from 3- to 2-ocean female fish, especially in the years when mesh sizes were larger than 5½ inches. A mesh size experiment conducted in 1928 by the Bureau of Fisheries illustrates this point. The log ratio of catches made with nets of 5½-inch and 6-inch mesh sizes are plotted by centimeter groups in Figure 6 and form an expected straight line. The aberrant points toward the upper size range are due to a much larger sampling error because of the very few fish present at these sizes.

The essential element of an escapement is not the total number of fish present but the potential egg deposition they represent. During the first period of the Nushagak fishery, when net sizes ranged from 6¼ to 5¾ inches, escapements of the same numerical magnitude as in later years must have represented a substantially higher potential egg deposition since a much higher proportion of 3-ocean females was included in those escapements than in years with 5½-inch mesh size. On the average, 3-ocean females produce 650 eggs more per female than 2-ocean fish. The mean fecundity of these two groups are 3,639 and 4,290 eggs, respectively (Mathisen, 1962).

This net selection has another, more intangible aspect. Not only is fecundity greater in the larger 3-ocean females, but egg size is also a function of the size of the females (Mathisen, 1962). Thus there may be a higher survival of the progeny in this case than from eggs produced by 2-ocean females in the same environment. This concern was expressed in 1927 by

TABLE 9.—Summary of age in the commercial catches of sockeye salmon in Nushagak.

Sex	Period	Age-groups					No. of years sampled	2-ocean	3-ocean
		1.2	2.2	1.3	2.3	Others			
Male	1912-1919	21.32	8.66	57.08	11.11	1.83	6	21.98	68.19
	1925-1945	35.77	8.95	48.16	5.38	1.59	18	44.82	53.54
	1946-1966	42.19	9.55	40.77	4.12	3.37	21	51.74	44.89
Female	1912-1919	27.11	9.80	51.25	11.08	0.76	6	36.91	62.33
	1925-1945	25.42	7.05	60.48	4.97	1.43	18	32.47	65.45
	1946-1966	27.12	5.91	56.62	5.57	4.78	21	33.03	62.19

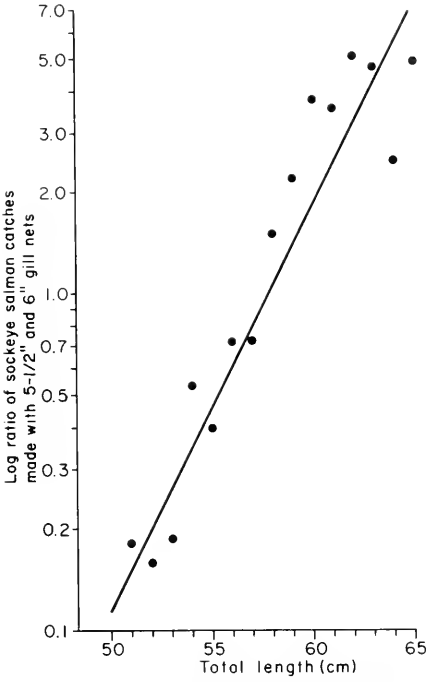

FIGURE 6.—Log ratio of catches made in Nushagak, 1928, by 6- and 5½-inch gill nets. Males and females combined by centimeter groups.

Gilbert, who wrote in a letter to Commissioner H. O'Malley:

> As a result of this screening process, we are selecting for breeding purposes predominantly the younger or less robust members of the colony, those that are dwarfed by reason of early maturity or lack of growth vigor. The effect of such continued breeding from the least fit of the community must result, it would seem, in the gradual impoverishment of the race and the reduction in size and value of the individuals composing it.

The inference may be made that the observed shifts in run strength and productivity in Nushagak are associated with changes in gear selectivity, by a shift to smaller net sizes over the years which reduced the potential egg deposition rather than the numerical size of the escapements. Such an explanation would be most appropriate for the transfer from the first to the second major period of the Nushagak fishery in 1919. But this argument loses some strength when other sockeye salmon systems outside Bristol Bay are considered.

The given description of the Nushagak fishery and reduction of reproductive rate are almost identical to that described for the Karluk sockeye fishery by Rounsefell (1958). A major portion of the Karluk River catches were taken in beach seines at the river mouth or in adjacent traps, both of which are nonselective for size. Gill nets never played a dominant part in harvest of the Karluk sockeye salmon. In spite of the absence of gear selective for size, a selection from the middle part of the run was present (Thompson, 1951).

The Chignik fishery offers another example. Recently Dahlberg (1968) and others before him have pointed out the almost identical catch curves for the Chignik and Nushagak fishery. In the Chignik fishery one can distinguish three major production levels, and the relative position of these are the same as observed in Nushagak (Figure 7). The only difference is that the fall from an initial high production level to an intermediate one came a few years later, 1926-1927, in the case of the Chignik fishery. Traps were for a long time the principal fishing

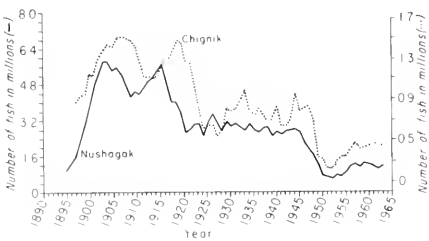

FIGURE 7.—Comparison of catches of sockeye salmon in the Chignik and Nushagak fisheries. Curves smoothed by a moving average of 5's.

gear at Chignik, later supplemented with seines, which today are the only gear operated. Gillnetting never became effective at Chignik because of the clear water there and the narrow channel. But as in the case of the Karluk fishery, there has been a selection and domination of certain races (Dahlberg, 1968).

In three important sockeye-producing systems, the historical development has been the same and resulted in a continuous decline of return per spawner. Dahlberg sees the cause as differential fishing mortality on the Chignik Lake and Black Lake races with an overfishing on the latter ones. Rounsefell (1958) suspects the effect of predation and stabilization of predator populations in the absence of the former large contrast between off and peak years as the principal cause. The evidence presented for the Nushagak fishery points to various effects of net selectivity as a major contributing factor. Viewed by themselves, each of the presented explanations may appear plausible, but the almost identical happenings in three unrelated systems suggest that a common underlying cause also may be operating.

In all three cases the decline started two decades or so after large-scale commercial harvest had been in operation. Provided the initial rate of reproduction remained the same the spawning stock or the potential egg deposition was sufficient to maintain the runs, or rebuild them after stringent regulations were put into effect.

One might conclude that the primary production of the nursery areas, which all are oligotrophic lakes in the three mentioned systems, started a slow decline from the moment fishing began, and the enrichment from salmon carcasses was substantially reduced relative to the situation which prevailed prior to commercial harvest.

Clearly, a hypothesis of this type cannot be demonstrated from the data presented. Rather, conclusive evidence must be sought from other sources. One is descriptive and involves a study of the sedimentation rates in prefishing years and in recent ones from bottom cores. Great changes in basic lake productivity should be reflected in the yearly sedimentation rate of diatom shells. Donaldson (1967) has demonstrated that changes in escapement level are indicated by phosphorus content of corresponding bottom sediments.

Experimental evidence on the role of the biogenic enrichment from salmon carcasses can be obtained from a lake fertilization similar in content, volume, and mode of dispersion to that provided by the dying spawners themselves.

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CHANGES IN CATCH AND EFFORT IN THE ATLANTIC MENHADEN PURSE-SEINE FISHERY 1940-68

WILLIAM R. NICHOLSON[1]

ABSTRACT

The catch, number of vessel weeks, and catch per vessel week in the Atlantic menhaden fishery increased during the 1950's. During this period fishing methods improved and the efficiency of vessels increased. Improvements included use of airplanes for spotting schools, aluminum purse boats, nylon nets, power blocks, and fish pumps for catching and handling fish, and larger and faster carrier vessels that could range farther from port. The catch and catch per vessel week began declining north of Chesapeake Bay in the early 1960's. By 1966, fish north of Chesapeake Bay had become so scarce that plants either closed or operated far below their capacity. In Chesapeake Bay the number of vessel weeks increased, and the catch and catch per vessel week decreased through the early and mid 1960's. Variations in catch, effort, and catch per unit of effort showed no trends in the South Atlantic. The annual mean number of purse-seine sets per day varied in different areas and ranged from about 2.0 to 4.5. The annual mean catch per set ranged from about 11 to 25 metric tons.

Catch and effort statistics are important in evaluating and managing any fishery. They may be used in measuring changes in actual or apparent abundance, estimating population sizes and mortality rates, and determining optimum fishing rates.

When investigations of the Atlantic menhaden (*Brevoortia tyrannus*) fishery were begun in 1955 by the Bureau of Commercial Fisheries, provisions were made for collecting and compiling catch and effort statistics. The number and locations of daily purse-seine sets were obtained from logbooks placed aboard vessels at the beginning of the fishing season, and daily catches of individual vessels were copied from plant records.

The objectives of the present study were: (1) to analyze logbook data and vessel landing records to determine differences and changes in the number of purse-seine sets, mean number of sets per day, and the mean catch per set, both between and within geographical divisions of the fishery, (2) to develop a method of measuring fishing effort, and (3) to document changes that have occurred in the fishery.

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BRIEF HISTORY OF THE FISHERY

Atlantic menhaden are found from central Florida to Nova Scotia and at one time or another have been exploited over most of this range. Fishing began in the early part of the 19th century in waters off Massachusetts and Maine. Following improved methods of fishing, extracting oil, and processing meal, the fishery expanded in this area in the latter part of the 19th century. When the scarcity of menhaden in waters north of Cape Cod caused the collapse of the fishery in that area, about 1895, the industry shifted to the Middle and South Atlantic coast. By the 1930's processing plants were located in approximately the same areas where they occur today (Figure 1).

Although in some areas pound nets capture menhaden incidentally with other species, purse seines catch nearly all of the fish that are reduced for meal and oil.

Purse seining began in the late 19th century and by present standards was inefficient and laborious. Purse boats were rowed and carrier vessels were sailed. Gradually, sailing vessels were replaced by larger, coal burning steam ships, purse boats were equipped with gasoline

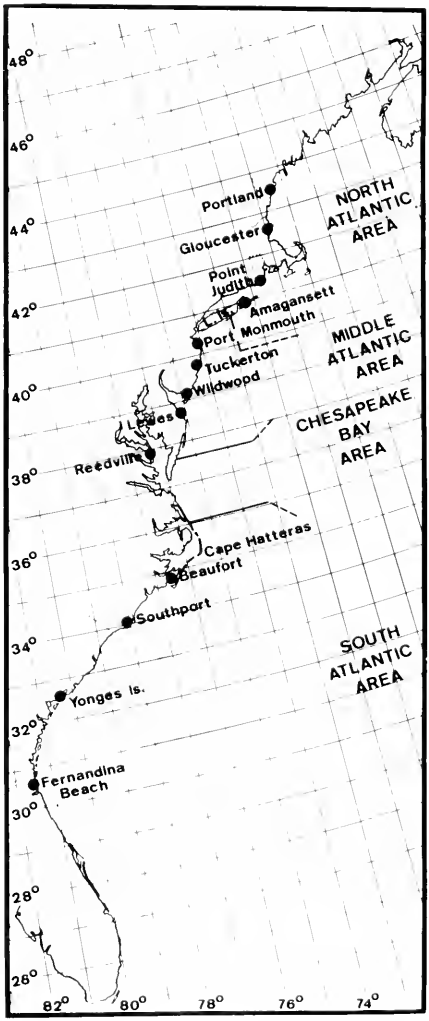

FIGURE 1.—Ports and major fishing areas, Atlantic menhaden fishery.

engines, and seines were made larger. Following World War I, diesel and gasoline engines gradually replaced steam engines in the carrier vessels. Methods of catching and processing menhaden, however, changed very little between World Wars I and II.

After World War II the increased demand for fish meal and oil initiated changes in the industry. Numbers and sizes of vessels increased, methods of fishing changed, processing facilities expanded, and processing efficiency increased.

A major change in fishing methods occurred in 1946 when airplanes were introduced to locate concentrations of fish. Plant operators found this practice so successful that they rapidly added more planes in following years (Table 1). Initially, airplanes scouted wide areas and directed vessels to places where menhaden were abundant. Later, after captains were given portable radios, the airplane pilot directed the actual setting of the net. Since about 1950 airplanes have been an integral part of fishing operations (Robas, 1959; June, 1963).

Fish pumps, initially installed on carrier vessels in 1946, were the first significant advance

TABLE 1.—Number of airplanes used in the Atlantic menhaden fishery.[a]

Year	North Atlantic	Middle Atlantic	Chesapeake Bay	South Atlantic	Total
1945	0	0	0	0	0
1946	0	1	1	0	2
1947	0	7	1	2	10
1948	1	8	1	2	12
1949	1	11	1	2	15
1950	1	10	1	3	15
1951	1	10	1	3	15
1952	2	11	1	4	18
1953	4	11	1	4	20
1954	4	12	3	4	23
1955	5	15	5	4	29
1956	6	15	8	4	33
1957	8	15	9	4	36
1958	8	17	10	4	39
1959	7	17	12	5	41
1960	5	16	7	4	32
1961	6	16	8	4	34
1962	6	16	9	3	34
1963	5	16	11	3	35
1964	4	18	13	4	39
1965	3	6	18	4	31
1966	1	4	18	4	27
1967	0	2	16	4	22
1968	1	2	16	4	23

[a] Exact data are not available for the North Carolina fall fishery. Estimates indicate that 20 to 25 were used each year after about 1955.

in fishing methods after World War II (Robas, 1959). Pumping fish directly from the purse seine to the hold replaced the time-consuming method of brailing and left more time for scouting and making additional sets. By 1955 nearly all vessels were equipped with fish pumps (Table 2).

Before fish in the seine can be pumped or brailed aboard the carrier vessel, they must be concentrated, or "hardened-up." This can be done by crewmen in the purse boats pulling in the net by hand, but it is a laborious process that requires approximately 22 men. A mechanical device, or "power block," for "drying-up" the net, used experimentally in 1955, became operational in 1956 (Schmidt, 1959a). Its use reduced the crew by 6 to 10 men and the average time to "harden-up" the fish by about 6 min (Schmidt, 1959a, 1959b), and enabled the crew to retrieve the net quickly if the fish were missed. Powerblocks were used extensively for the first time in 1958 and by 1966 were installed on nearly all vessels from Long Island southward (Table 2).

Large sets are sometimes lost when the net cannot be raised manually or mechanically to concentrate the fish so that they may be pumped. But the pump head, if positively charged with electricity, becomes an electrode that attracts and concentrates menhaden without the necessity of raising the bunt (Kreutzer 1959). Such a device, commonly called a "fish shocker," was first installed on vessels in 1956, but its use did not spread beyond the Middle and North Atlantic areas. By 1966 it had fallen into disuse (Table 3).

Beginning in 1954, nylon nets gradually replaced cotton or linen nets (Table 3). Although more expensive initially, nylon nets last longer and do not split or tear when filled with fish as other nets sometimes do.

Aluminum purse boats began replacing wooden purse boats in 1957 (Table 3). Being lighter, more maneuverable, and more stable than wooden boats, they can encircle a school of fish easier

TABLE 2.—Percent of vessels equipped with fish pumps and power blocks in the Atlantic menhaden purse-seine fishery.

Year	Middle Atlantic[a]		Chesapeake Bay		South Atlantic	
	Fish pumps	Power blocks	Fish pumps	Power blocks	Fish pumps	Power blocks
1946-49	0	0	10-20	0	0	0
1950	6	0	24	0	0	0
1951	13	0	25	0	0	0
1952	32	0	31	0	0	0
1953	44	0	22	0	9	0
1954	48	0	25	0	10	0
1955	81	0	90	0	11	0
1956	78	2	92	0	23	0
1957	91	2	88	16	23	0
1958	96	100	100	57	25	0
1959	100	100	100	68	45	0
1960	100	100	100	95	45	0
1961	100	100	100	74	45	0
1962	100	100	100	66	50	31
1963	100	100	100	83	69	31
1964-68	100	100	100	100	64-74	42-69

a Includes Amagansett from North Atlantic Area

TABLE 3.—Percent of vessels equipped with fish shockers, nylon nets, and aluminum purse boats in the Atlantic menhaden purse-seine fishery.

Year	Middle Atlantic[a]			Chesapeake Bay			South Atlantic		
	Fish shockers	Nylon nets	Aluminum purse boats	Fish shockers	Nylon nets	Aluminum purse boats	Fish shockers	Nylon nets	Aluminum purse boats
1954	0	2	0	0	0	0	0	0	0
1955	0	5	0	0	0	0	0	0	0
1956	47	19	0	0	4	0	0	0	0
1957	45	18	0	0	44	0	0	13	0
1958	85	100	66	0	82	4	0	14	0
1959	85	100	85	0	94	23	0	20	0
1960	82	100	82	0	100	32	0	45	0
1961	82	100	82	0	100	30	0	58	0
1962	82	100	89	0	100	63	0	81	33
1963	82	100	100	0	100	83	0	100	50
1964	78	100	100	0	100	100	0	100	50
1965	86	100	100	0	100	100	0	100	58
1966	0	100	100	0	100	100	0	100	69
1967	0	100	100	0	100	100	0	100	100
1968	0	100	100	0	100	100	0	100	100

a Includes Amagansett from North Atlantic Area.

and faster, and can operate more easily in rough seas.

Three jet-propelled purse boats were introduced in 1962. Adjustable jet nozzles on each end gave the boats excellent maneuverability, and there was no propeller or guard to entangle nets. They lacked the power to close up the seine rapidly, however, and were abandoned.

With the exception of airplanes for spotting, none of the improvements were adopted by vessels in the Point Judith, Gloucester, or Portland fleets. All vessels fishing from these ports were small to medium-sized otter trawlers that were converted to purse seining for only about 2 months during the summer.

DESCRIPTION OF THE FISHERY

The purse-seine season for menhaden extends from late spring through fall, but the time varies in different localities. South of Cape Hatteras, N.C., it begins in April or May and lasts until late December or early January. From Chesapeake Bay to the southern shore of Long Island it begins in late May and usually ends about the third week in October. North of Cape Cod the season lasts only from about late June to early September.

To facilitate summarizing and discussing annual changes in the fishery, June and Reintjes (1959) divided the range of Atlantic menhaden into four areas, the North Atlantic, Middle Atlantic, Chesapeake Bay, and South Atlantic (Figure 1). Although the boundaries are arbitrary, they were drawn to take advantage of natural separations in the fishing areas. Similarities in age and size composition of the catches, time and duration of fishing, and range of vessels from the home port tended to set each area apart. The North Carolina fall fishery, a specialized fishery that occurs only during November and December from Cape Hatteras to Cape Fear, is distinct from the summer fishery in the South Atlantic and was treated as if it were an area. This classification, which provides a convenient way of expressing statistics of the fishery, is used in the present analysis. Ports in the South Atlantic area are Fernandina Beach, Fla.; Yonges Island, S.C.; and Southport and Beaufort, N.C.; in the Chesapeake Bay area—Reedville, Va.; in the Middle Atlantic area, Lewes, Del.; and Wildwood, Tuckerton, and Port Monmouth, N.J.; in the North Atlantic area—Amagansett, N.Y.; Point Judith, R.I.; Gloucester, Mass.; and Portland, Maine; and in the North Carolina fall fishery—Beaufort and Morehead City, N.C.

A disadvantage of the fishery area concept is that all of the fish landed at a port in a particular area may not have been caught in that area. The problem is not serious, however, because vessels seldom fish in areas other than the one in which their home port is located. Port Monmouth vessels, which sometimes go through the East River to fish in western Long Island Sound, and Amagansett vessels, which sometimes fish off the northern New Jersey coast, contradict this general rule more often than do vessels at other ports.

The number and location of daily purse-seine sets each year from 1955 to 1966 were obtained from logbooks placed aboard vessels at the beginning of each fishing season. Port samplers were instructed to pick up copies of each page every 2 weeks, answer questions pertaining to the methods of keeping the logs, and stimulate interest of the pilots to keep the logs complete and up to date. From 25 to 100% of the boats fishing at each port kept logs. Although generally over 60% of the fleet was covered each year, many vessels did not keep complete records.

Daily landings of each vessel were copied from plant records. Although some records extended back as far as 1912, records at most plants were not available for years prior to 1940.

ANNUAL CATCH

No trends were evident in the annual catches in the South Atlantic area or North Carolina fall fishery, but the catches in the other three areas reflected an increase in fishing effort after 1945 and a decline in abundance after 1956 (Table 4). After reaching a peak in 1956 of 378,300 metric tons in the Middle Atlantic area and 98,500 tons in the North Atlantic area, the catch declined to 6,000 and 1,800 tons, respectively in 1966. In the Chesapeake Bay area the catch decreased from 196,800 metric tons in

1959 to 115,600 tons in 1966. In the North Atlantic area, the Point Judith, Gloucester, and Portland fleets, which began menhaden fishing about 1949, accounted for most of the increases between 1950 and 1960. Menhaden were not landed at Portland after 1957 or at Gloucester and Point Judith after 1962.

CALCULATION OF FISHING EFFORT

In any searching fishery where the sizes and types of vessels vary, the unit of fishing effort is difficult to define. Marr (1950) found a positive linear relation between mean catch per boat week and boat length in the Pacific sardine (*Sardinops sagax*) fishery off Oregon. He selected the modal boat-length group as a standard, calculated the catch per boat week of boats in this group for each year, and based his estimates of apparent abundance on this index. He was unable, however, to estimate total effort except by dividing the total catch of all vessels by the catch per unit of the standard group.

Silliman and Clark (1945), studying the Pacific sardine fishery off California, linked groups of identical vessels and estimated apparent abundance from the catch per boat week of these groups. They estimated the total effort by dividing the total catch by the catch per boat week of the linked group, assuming that the catch per boat week of the selected vessels was representative of the catch per boat week of the fleet, and using a base season for each of the three areas they studied. Recognizing the effect of differences in vessel size on catch per unit of effort, they used a standard multiple regression to estimate total effort in each area by relating in a single equation the length and horsepower of each vessel, the number of vessels, and the number of weeks.

Clark and Daugherty (1950) extended the study by Silliman and Clark through the 1948-49

TABLE 4.—Atlantic menhaden purse-seine catch by year and area.

Year	Area					Total
	North Atlantic	Middle Atlantic	Chesapeake Bay	South Atlantic	North Carolina fall fishery	
			thousands of metric tons			
1940	16.8	91.1	35.3	37.9	36.6	217.7
1941	33.5	104.1	60.2	45.2	34.9	277.9
1942	14.6	77.7	21.9	32.9	20.1	167.2
1943	9.8	96.8	42.1	59.7	28.8	237.2
1944	27.5	122.6	32.2	46.9	28.7	257.9
1945	34.0	136.4	35.1	58.5	31.9	295.9
1946	42.9	183.8	57.6	40.8	37.3	362.4
1947	44.2	185.8	81.2	34.2	32.9	378.3
1948	44.4	137.4	68.3	55.8	40.6	346.5
1949	52.2	149.8	62.8	59.3	39.7	363.8
1950	49.3	143.0	63.1	20.0	21.8	297.2
1951	51.0	168.6	56.1	54.6	31.1	361.4
1952	58.1	193.7	45.7	86.0	26.4	409.9
1953	59.7	363.2	77.8	52.8	39.7	593.2
1954	64.9	335.7	126.0	39.6	41.9	608.1
1955	83.3	317.6	132.7	43.4	64.4	641.4
1956	98.5	378.3	94.0	68.6	72.7	712.1
1957	83.5	304.5	126.4	36.4	52.0	602.8
1958	36.0	211.1	151.3	41.3	70.3	510.0
1959	66.0	250.9	196.8	63.1	82.3	659.1
1960	66.4	256.0	108.5	36.7	62.2	529.8
1961	58.6	274.6	128.7	44.1	69.9	575.9
1962	64.7	249.9	155.1	42.2	25.8	537.7
1963	35.2	111.7	104.0	34.2	62.8	347.9
1964	15.0	35.2	134.1	46.5	38.4	269.2
1965	11.9	45.8	126.1	36.7	52.9	273.4
1966	1.8	6.0	115.6	24.5	71.7	219.6
1967	0	17.1	91.1	34.1	51.2	193.5
1968	6.7	26.2	115.5	33.6	52.8	234.8

season. They also used linked groups of vessels, but simplified calculations by using catch per lunar month rather than catch per week.

June and Reintjes (1957), studying the menhaden fishery off Delaware Bay, used the linkage method to determine the catch per boat week for selected boats from 1939 to 1953. They estimated the total number of boat weeks by dividing the total catch by the catch per boat week.

In the yellowfin tuna (*Thunnus albacares*) bait-boat fishery in the eastern tropical Pacific, Shimada and Schaefer (1956) grouped vessels by carrying capacity. They computed the catch per days absence from port for each group and established one group as a standard. They standardized effort by dividing the catch per days absence of the standard group by the catch per days absence of each other group. Broadhead (1962) related the catch per day of bait boats to the catch per day of purse-seine vessels by using regression analysis.

Menhaden plant records, while showing the date and amount of fish landed by each vessel, do not list days when vessels fish and catch nothing, and do not indicate whether a catch represents 1 or more days' fishing. While vessels generally land their catch daily, quite often in the Middle and North Atlantic areas they land 2 or 3 days' catch at one time, particularly in late spring and early fall, a practice which has increased in recent years as fish have become scarce and daily catches smaller.

There is no satisfactory way of getting the complete daily history of each vessel. Even if port samplers recorded each vessel's daily activity, the records still would be incomplete because not all ports are sampled and because no ports were sampled prior to 1955. Logbook records also are incomplete. Any effective method of measuring effort, therefore, must use vessel landings as they are recorded at the plants.

Fortunately, menhaden vessels generally operate continuously throughout all or part of the fishing season and fish every day that weather permits, unless in port for repairs. Except in the North Carolina fall fishery, which lasts only 6 to 8 weeks, the number of days that bad weather prohibits menhaden fishing is relatively small and is relatively constant from year to year. Any time period, therefore, that assumes continuous fishing and accounts for unproductive fishing days should be a satisfactory unit of basic fishing effort. Because the vessel week satisfies these conditions and may be readily computed, it was selected as the basic unit.

Because variations in the catch per unit of effort among vessels may necessitate adjusting the basic unit to a common standard, the relative efficiency of vessels fishing from each port was examined.

No clear correlation could be shown between catch per week and vessel length or weight, so the relation between mean catch per week and vessel carrying capacity was explored. Carrying capacity, determined for each vessel by averaging the 10 largest catches for 3 consecutive years, ranged from about 100 to 350 short tons (90-317 metric tons). Vessels were grouped, according to their carrying capacity in short tons, into six classes:

Class	Carrying capacity
1	<141
2	141-180
3	181-220
4	221-260
5	261-300
6	>300

The relative efficiency of vessels at each port was examined by plotting the mean catch per week of each vessel and by plotting the catch per week against carrying capacity.

In the South Atlantic area all vessels were class 3 at Fernandina Beach, Yonges Island, and Southport, and class 1 or 3 at Beaufort. Variation in the catch per week among vessels was evident at all ports, but there was no distinct tendency for any group to have larger or smaller catches per week than another.

Until about 1963 nearly all vessels in Chesapeake Bay were class 3, although a few were class 2, 4, or 5. After 1961 the number of class 5 vessels increased. Although the large capacity vessel tended to have greater mean catches per week than small capacity vessels, the variation was extreme among all vessels, both between and within years. As the catch per week declined after 1961, the variation between vessels of small and large capacities decreased.

In the Middle Atlantic area vessels ranged from class 2 to 6, but no more than two classes occurred at any port. At Port Monmouth and Tuckerton, class 6 vessels did not show substantially greater catches per week than class 5 vessels. Class 5 vessels at Lewes clearly had greater catches per week than class 3 vessels, while class 5 vessels at Wildwood had greater catches per week than class 2 vessels.

Because the increases in the catch per vessel week that accompanied the increases in vessel carrying capacity were small and inconsistent and the variability between ports was great, no vessel class was designated as a standard for the fishery. Effort was simply left unadjusted at all except five ports—Lewes, Wildwood, Point Judith, Gloucester, and Portland.

Effort at Lewes and Wildwood was adjusted because the differences in the catch per unit of effort between the two classes at each port were large. At these ports the 10-year mean of the ratio of the catch per week of the group of larger vessels to that of the group of smaller vessels was computed for 1950-59. Annual effort of the smaller vessels was adjusted by multiplying the total number of weeks fished each year by the mean ratio, 0.610 for Wildwood and 0.573 for Lewes.

Effort at the other three ports was adjusted because many of the vessels, which were small to medium-size otter trawlers temporarily converted to purse seiners during the summer, fished intermittently, usually only when menhaden were plentiful. Because effort could not be measured very precisely under these conditions, it was estimated in terms of Amagansett units by dividing the annual catches at these ports by the mean catch per week of Amagansett vessels.

Most menhaden vessels were class 3, 4, or 5. At most ports the relative proportions of one class to another changed very little each year and the number of vessels remained fairly constant (Table 5). Under such conditions the number of vessel weeks, with minor adjustments, was as precise an estimate of total fishing effort as was possible to obtain. Various other adjustments might have been made, but with doubtful improvement in the overall estimate of fishing effort.

Henceforth, vessel weeks will refer to units of fishing effort and will include these adjustments.

NUMBER OF VESSEL WEEKS

After World War II ended in 1945, the number of vessel weeks rose sharply in the Chesapeake Bay, Middle Atlantic, and North Atlantic areas (Table 6). The increase resulted from the addition of vessels in all areas and from an increase in the number of weeks that plants in the North and Middle Atlantic operated.

After 1959 in the North Atlantic and 1962 in the Middle Atlantic, the number of vessel weeks dropped sharply. Much of the decrease in the North Atlantic between 1959 and 1962 can be attributed to a reduced number of converted trawlers at Portland, Gloucester, and Point Judith, where no menhaden were landed after 1962. After 1962 the number of vessels at Amagansett also declined. Reduced effort in the Middle Atlantic after 1962 was due to a decrease in the number of vessels. The Tuckerton plant and one of the Lewes plants closed during the 1964 season and never reopened. The Wildwood plant operated only a few weeks each year after 1964, and the boats were transferred to plants in Chesapeake Bay. The remaining plant at Lewes closed after the 1965 season.

Effort in the Chesapeake Bay area fluctuated between approximately 300 and 400 vessel weeks from 1944 to 1954; thereafter it generally increased (except when fishing was restricted in 1960 because of a poor market) until about 800 vessel weeks were reached in 1964-66. Additional vessels accounted for most of the increase through 1963. In 1964-68, fishing terminated approximately 7 weeks later (mid-November) than in previous years.

In the South Atlantic area, effort fluctuated between 245 and 530 vessel weeks from 1941 to 1968. Although some fluctuation was due to variation in the length of the fishing season, particularly in Florida, most was due to variations in the number of vessels. The annual number of vessels, and vessel weeks, generally was less from 1960 to 1968 than in previous years.

In the North Carolina fall fishery, the number of vessel weeks varied from 97 to 457 and

TABLE 5.—Number of menhaden vessels fishing at Atlantic coast ports, by class capacity.

Year	Amagansett, N.Y.		Port Monmouth, N.J.		Tuckerton, N.J.		Lewes, Del.		Wildwood, N.J.		Reedville, Va.				Beaufort, N.C.			Southport, N.C.		Fernandina Beach, Fla. and Yonges Is., S.C.
	Class		Class		Class		Class		Class		Class				Class			Class		Class
	5	6	5	6	5	6	3	5	2	5	2	3	4	5	1	2	3	2	3	3
1940	a	a	a	a	0	0	15	4	3	0	2	7	1	0	10	4	0	4	0	a
1941	9	0	a	a	0	0	12	3	5	0	2	12	1	0	6	1	0	5	0	15
1942	9	0	a	a	0	0	16	3	3	0	2	12	1	0	6	1	0	3	0	10
1943	5	0	a	a	0	0	12	1	3	0	1	8	0	0	5	0	0	2	0	15
1944	6	0	a	a	0	0	20	4	3	0	1	9	1	0	6	0	0	3	0	13
1945	6	0	a	a	4	0	10	5	2	0	1	10	1	0	5	0	0	3	0	12
1946	7	0	a	a	5	0	12	7	2	0	1	8	1	0	6	0	0	3	0	8
1947	8	0	a	a	5	0	14	6	2	0	1	13	1	0	5	0	1	3	0	8
1948	9	0	a	a	5	0	20	11	3	0	2	14	1	0	9	2	2	3	0	12
1949	7	2	a	a	6	0	15	10	3	0	1	13	1	0	7	1	2	3	3	14
1950	7	2	a	a	6	0	12	11	3	1	1	13	1	2	5	2	3	3	3	8
1951	8	2	a	a	6	0	12	13	2	2	1	13	1	1	5	2	1	2	3	9
1952	7	2	a	a	6	0	7	11	2	2	1	10	1	1	6	1	2	3	5	10
1953	7	2	a	a	5	1	10	14	3	3	1	16	1	0	6	1	2	3	5	12
1954	7	2	a	a	5	1	10	12	4	3	2	17	1	0	6	1	2	2	5	11
1955	8	2	8	2	5	1	9	16	3	4	2	16	1	1	6	11	2	1	6	14
1956	9	2	7	3	4	2	6	18	2	5	3	19	1	1	6	1	3	1	7	12
1957	8	2	6	4	4	2	6	16	3	5	4	18	1	2	6	1	3	0	7	14
1958	8	2	6	4	4	2	5	15	2	6	4	19	1	4	6	1	3	0	7	9
1959	8	2	6	4	4	2	4	17	2	6	5	21	1	4	7	0	3	0	8	7
1960	8	2	6	4	4	2	4	17	2	8	2	16	1	3	6	0	3	0	8	3
1961	8	2	6	4	4	2	4	17	2	8	3	17	1	2	6	0	3	0	8	3
1962	8	2	6	4	4	2	4	17	2	8	3	17	1	8	5	0	3	0	4	3
1963	8	2	6	4	4	2	3	18	2	7	3	18	1	14	5	0	3	0	5	3
1964	7	2	3	4	4	2	0	15	2	7	2	16	1	19	5	0	3	0	5	3
1965	4	2	1	5	0	0	0	7	0	b	2	13	1	22	5	0	3	0	5	6
1966	3	2	1	4	0	0	0	5	0	b	2	8	1	25	5	0	3	0	3	5
1967	0	0	1	3	0	0	0	0	0	b	0	8	1	23	6	0	2	0	3	5
1968	0	2	1	3	0	0	0	0	0	b	0	6	1	18	6	0	2	0	3	5

a Records not available.
b Variable.

TABLE 6.—Number of vessel weeks per season in the Atlantic menhaden fishery, by year and area.

Year	Area					Total
	North Atlantic	Middle Atlantic	Chesapeake Bay	South Atlantic	North Carolina fall fishery	
1940	a	337	329	a	a	a
1941	141	392	417	506	227	1,683
1942	89	323	251	376	194	1,233
1943	49	287	202	419	166	1,123
1944	84	397	296	316	224	1,317
1945	89	477	302	394	234	1,496
1946	132	528	294	343	291	1,588
1947	134	552	418	322	333	1,759
1948	130	675	405	430	288	1,928
1949	156	691	385	473	457	2,162
1950	155	614	403	322	187	1,681
1951	157	676	369	379	222	1,803
1952	150	580	333	474	220	1,747
1953	161	819	376	474	244	2,074
1954	189	838	408	488	262	2,185
1955	334	890	451	475	342	2,492
1956	298	888	466	530	391	2,573
1957	262	949	527	412	311	2,461
1958	227	734	559	354	380	2,254
1959	301	897	668	474	312	2,652
1960	280	854	410	292	163	1,999
1961	249	946	482	395	224	2,296
1962	264	990	582	327	97	2,260
1963	238	823	666	264	286	2,277
1964	134	376	803	277	249	1,839
1965	96	300	786	359	259	1,800
1966	79	87	795	254	220	1,435
1967	0	124	757	253	212	1,346
1968	23	113	601	245	246	1,228

a Records not available.

depended primarily on the number of vessels, the season generally lasting about 7 or 8 weeks.

CATCH PER VESSEL WEEK

Despite sharp fluctuations that occurred annually, there were pronounced trends in the catch per vessel week in three areas (Table 7). In the North Atlantic area, the catch per vessel week remained at a high level through 1957, dropped sharply in 1958 and continued to decline thereafter. From a peak of 385 metric tons per week in 1952, it dropped to 23 in 1966. The high figure for 1968 (292) reflects the fact that two vessels, one fishing from late June until mid-October and another during August and early September, caught most of the fish available.

The most significant changes occurred in the Middle Atlantic and Chesapeake Bay areas. From 348 metric tons per week in 1946, the catch per vessel week in the Middle Atlantic area dropped to 203 in 1948, and thereafter rose steadily, attaining 444 tons in 1953. From 1954 to 1957 it remained high, between 320 and 426 tons. Between 1958 and 1961, it declined relative to the previous 4 years, but still remained between 279 and 299 tons. It dropped to 253 tons in 1962, and 70 tons in 1966. By contrast, the catch per vessel week in the Chesapeake Bay area was low from 1943 to 1953, fluctuating between 109 and 207 tons, and high from 1954 to 1962, fluctuating, except for 1956, between 239 and 309 tons. In 1963 the catch per week dropped to 156 tons and then continued a downward trend.

In the South Atlantic the catch per vessel week showed no trends. The figures generally ranged from about 86 to 136 metric tons, with extreme fluctuations of from 39 to 180 tons.

In the North Carolina fall fishery the catch per vessel week from 1941 to 1954 fluctuated

TABLE 7.—Mean catch of Atlantic menhaden per vessel week, in metric tons, Atlantic menhaden fishery.

Year	Area				
	North Atlantic	Middle Atlantic	Chesapeake Bay	South Atlantic	North Carolina fall fishery
1940	a	273	107	a	a
1941	237	266	144	89	154
1942	164	240	88	88	104
1943	200	337	208	142	173
1944	327	309	109	149	128
1945	383	286	116	148	136
1946	325	348	196	119	128
1947	329	336	194	106	98
1948	342	203	169	130	141
1949	335	217	163	125	87
1950	318	233	157	62	117
1951	325	249	137	144	140
1952	387	333	136	181	120
1953	371	444	207	111	162
1954	343	401	309	81	160
1955	249	357	294	91	188
1956	330	426	201	130	186
1957	319	320	239	88	167
1958	159	288	270	117	185
1959	219	279	295	133	263
1960	237	299	265	126	381
1961	235	290	267	111	312
1962	245	253	267	129	266
1963	148	135	156	130	220
1964	112	93	167	168	154
1965	124	152	161	102	203
1966	23	69	145	96	326
1967	0	138	121	135	241
1968	292	232	192	137	215

a Records not available.

between 87 and 173 metric tons and averaged 132 tons, and from 1955 to 1968 fluctuated between 156 and 381 and averaged 239 tons. The increased use of airplanes and other improvements in fishing methods, rather than any increases in the abundance of menhaden, probably were responsible for the large catches per vessel week in the later years.

While the catches per vessel week were lower for ports in the South Atlantic area than for ports in the Middle Atlantic, the variation between ports in each area was of about the same magnitude (Table 8). In the South Atlantic the figures for Southport and Fernandina Beach were about equal to each other but higher than for Beaufort. In the Middle Atlantic the catch per vessel week usually was highest at Tuckerton.

The monthly catch-per-vessel-week figures were computed for each area, but they showed no consistent trends or variation worth noting.

NUMBER OF PURSE-SEINE SETS

The number of purse-seine sets was estimated from logbooks and reduction plant records by the formula:

$$S_t = L_t(S_1 / L_1)$$

where:

S_t = number of estimated monthly sets,
S_1 = number of sets from logbooks,
L_1 = number of days for which number of sets is known,
L_t = total number of landings days from plant records.

Vessels at each port were stratified by months and by loading capacity, on the assumption that the number of sets per day varied with both time and capacity. The number of monthly sets was estimated for vessels in each stratum.

TABLE 8.—Mean catch of Atlantic menhaden per vessel week, in metric tons, landed at ports in the South and Middle Atlantic areas.

Year	Fernandina Beach, Fla., and Yonges Is., S.C.	Southport, N.C.	Beaufort, N.C.	Lewes, Del.	Wildwood, N.J.	Tuckerton, N.J.	Port Monmouth, N.J.
1940	a	73	27	255	165	b	318
1941	106	88	36	292	211	b	257
1942	109	84	34	261	243	b	217
1943	170	119	51	337	309	b	344
1944	170	148	107	319	269	b	302
1945	134	164	97	331	309	198	256
1946	121	181	80	413	331	306	278
1947	73	180	103	382	234	343	252
1948	129	158	118	189	210	216	226
1949	78	207	110	238	200	213	186
1950	71	54	57	256	164	270	188
1951	193	82	70	266	140	279	233
1952	247	154	75	367	177	412	259
1953	140	98	75	444	505	524	374
1954	100	93	72	438	359	457	327
1955	85	107	80	404	333	433	258
1956	138	148	103	436	346	518	399
1957	86	104	73	301	255	368	377
1958	63	193	74	366	268	221	194
1959	194	161	70	301	253	252	273
1960	184	143	86	346	259	278	259
1961	157	132	64	332	259	258	258
1962	180	133	92	247	240	254	269
1963	149	108	127	115	150	137	155
1964	129	227	140	34	80	61	56
1965	105	137	69	131	103	b	187
1966	137	67	72	50	238	b	48
1967	173	172	192	b	171	b	122
1968	122	260	189	b	175	b	261

a Records not available.
b Plant closed.

Monthly totals at each port were obtained by summing the estimates for each stratum, seasonal totals by summing the monthly estimates, and area totals by summing the totals of each port. The mean number of sets per day for either month or season was calculated by dividing the total number of estimated sets by the total number of fishing days.

Because of the difficulty of maintaining good logbook records in recent years, the analysis was not continued beyond 1966. By that time little fishing was done north of Chesapeake Bay.

At ports where more than one size class of vessels fished, the larger vessels generally averaged slightly more sets per day than the smaller ones (Table 9). The differences were greater at ports where the vessel classes were not adjacent (Lewes and Wildwood) than they were at ports where the vessel classes were adjacent (Amagansett, Port Monmouth, and Tuckerton). Because data were insufficient to calculate the mean catch per set for each vessel class in Chesapeake Bay, the data were combined for classes 2 and 3, and 4 and 5. After 1964 the lack of data made meaningful comparisons impossible.

The slightly greater mean number of sets per day for the larger vessels may reflect the ability of these vessels to steam faster and range farther from their home port, and to carry more fish when fully loaded. More than likely, these figures reflect the ability and aggressiveness of the vessel captains, since the better ones generally are assigned to the larger vessels.

The annual or monthly number of sets (Table 10) reflected the abundance of fish and the amount of fishing effort. Excluding the North Carolina fall fishery, the most sets per season through 1963 were usually made in the Middle Atlantic area and the fewest sets in the South Atlantic. After 1963, following the drastic decline of the fishery and the decrease in effort in the Middle and North Atlantic areas, the number

TABLE 9.—Mean number of purse-seine sets per day, Atlantic menhaden fishery, by port and vessel class.

Year	Amagansett, N.Y.		Port Monmouth, N.J.		Tuckerton, N.J.		Lewes, Del.		Wildwood, N.J.		Reedville, Va.	
	Class		Class		Class		Class		Class		Class	
	5	6	5	6	5	6	3	5	2	5	2-3	4-5
1955	2.49	2.91	3.23	3.71	--	--	3.46	3.93	3.07	3.93	--	--
1956	2.82	2.88	3.43	4.03	4.41	4.68	3.38	3.98	3.15	3.99	--	--
1957	3.34	3.17	3.98	4.00	4.44	4.54	3.67	4.52	3.22	3.75	4.56	5.25
1958	2.63	2.83	3.26	2.71	2.78	3.67	3.79	3.07	--	--	4.12	3.76
1959	2.72	3.06	3.75	3.58	3.86	4.21	3.19	3.92	2.88	3.43	4.40	4.84
1960	2.79	2.81	3.69	3.54	4.29	4.57	--	4.84	3.61	3.96	3.86	4.38
1961	3.00	3.35	3.40	3.85	3.98	3.75	3.28	4.21	3.07	3.50	3.92	4.40
1962	2.78	3.29	3.49	3.85	3.49	3.61	3.53	3.46	2.74	3.09	2.86	3.42
1963	2.51	3.03	2.60	3.17	3.16	3.27	2.14	3.34	2.96	2.95	3.09	3.39
1964	2.30	2.91	2.41	2.65	--	3.85	--	--	--	--	2.95	3.62
Mean	2.74	3.02	3.32	3.51	3.80	4.02	3.31	3.92	3.09	3.58	3.72	4.13
Difference	0.28		0.19		0.22		0.61		0.49		0.41	

of sets in Chesapeake Bay, reflecting the increase in effort, was more than double the number in any other area.

There also were differences in the mean number of sets per day between areas (Table 11). Generally, the greatest number of sets per day was made in Chesapeake Bay, where vessels averaged about 0.10 set per day more than vessels in the Middle Atlantic. The fewest sets per day were made in the South Atlantic, where the tendency of schools to disappear by midday limited fishing to the forenoon, and in the North Carolina fall fishery, where the huge schools of fish enabled the vessels to load with relatively few sets.

CATCH PER SET

The mean catch per set varied monthly and annually in each area (Table 12). In all areas except the North Atlantic, it tended to be smaller during the middle part of the season than during the early or later part. In the Middle and North Atlantic areas, it averaged 9 tons more in October than in any other month. Annually, it fluctuated randomly in all areas except the Middle Atlantic, where it decreased after 1962.

Since purse seines tend to capture an entire school, the mean catch per set is an estimate of mean school biomass.

The school biomass appears to increase as the average age of the fish constituting the school increases. The catch per set in the North Atlantic, where 3-year and older fish constitute the bulk of catch, was higher than in the South Atlantic and Chesapeake Bay, where 1- and 2-year-old fish compose most of the catch (Nicholson and Higham, 1964). In the South Atlantic in 1960 and 1961, and in Chesapeake Bay in 1958, 1960, and 1961, when 2- rather than 1-year-old fish composed an unusually high percentage of the catch (Nicholson and Higham, 1964), the mean catch per set was relatively high. In the Middle Atlantic area in 1955 and 1956, when the catch contained a large percentage of fish older than age 2 (June and Reintjes, 1959, 1960), the mean catch per set was relatively high. Both the mean catch per set and the average age were low in the North Atlantic in 1957 and 1958. In the Middle Atlantic both the average age and the mean catch per set tended to decrease from June to September and then increase sharply in October, while in the North Atlantic both tended to increase from June to October.

Except for the South Atlantic area, where the disappearance of schools by midday limits the number of sets, the mean number of sets per day (Table 11) and the mean tons per set (Table 12) were inversely correlated, implying that fewer sets were necessary to load a vessel in areas where the school biomass was large, that schools became more numerous as their biomass decreased, or that heavy fishing pressure tended to keep school size small.

Relative abundance also appears to influence school biomass, but the relationship is not clear. When the catch per vessel week, a measure of

TABLE 10.—Estimated number of purse-seine sets in the Atlantic menhaden fishery, by year, month, and area.

Area	Year	Month							Total	North Carolina fall fishery
		Apr.-May	June	July	Aug	Sept.	Oct.	Nov.		
South Atlantic and North Carolina fall fishery	1955	640	1,422	716	303	77	196	0	3,354	1,477
	1956	1,421	817	463	572	411	197	0	3,881	2,358
	1957	606	761	435	564	315	85	0	2,766	1,556
	1958	704	708	574	365	561	118	0	3,030	2,354
	1959	812	1,260	1,129	847	506	225	0	4,779	1,827
	1960	126	506	590	847	131	105	0	2,305	1,408
	1961	310	512	420	512	457	131	0	2,342	1,316
	1962	432	648	454	573	806	259	0	3,172	2,568
	1963	513	354	380	606	449	140	0	2,442	2,121
	1964	403	634	540	496	216	--	0	2,289	1,412
	1965	270	531	392	393	309	246	0	2,141	1,826
	1966	120	298	257	387	235	62	0	1,359	1,603
Chesapeake Bay	1955	--	--	--	--	--	--	--	--	
	1956	0	2,025	1,485	2,444	1,361	376	0	7,691	
	1957	0	2,697	2,501	2,880	2,292	762	0	11,132	
	1958	0	1,916	2,093	2,208	2,423	550	0	9,190	
	1959	0	3,060	3,036	2,914	2,657	1,328	0	12,995	
	1960	0	1,450	1,468	1,878	1,525	899	0	7,220	
	1961	0	2,376	1,713	1,874	1,257	802	0	8,022	
	1962	0	1,306	2,751	1,346	1,491	1,227	0	8,121	
	1963	0	2,150	1,374	1,567	1,411	979	0	7,481	
	1964	0	2,253	1,798	1,925	1,747	1,334	1,037	10,094	
	1965	0	1,927	1,137	1,912	1,633	1,533	795	8,937	
	1966	0	1,473	1,484	2,460	1,386	1,550	888	9,241	
Middle Atlantic	1955	0	3,857	3,483	2,693	2,304	920	0	13,257	
	1956	0	3,740	3,258	4,264	2,130	950	0	14,342	
	1957	0	3,589	3,991	4,637	2,641	2,045	0	16,903	
	1958	0	1,216	2,352	3,098	2,919	243	0	9,828	
	1959	0	2,884	3,496	3,184	2,448	805	0	12,817	
	1960	0	2,894	3,889	4,707	2,162	1,556	0	15,208	
	1961	0	3,529	3,780	5,085	2,233	888	0	15,515	
	1962	0	5,243	1,987	2,732	1,839	1,088	0	12,889	
	1963	0	3,086	1,952	2,083	1,025	618	0	8,764	
	1964	0	1,035	832	663	576	111	0	3,217	
	1965	0	1,083	733	937	432	152	0	3,337	
	1966	0	359	168	162	63	--	0	752	
North Atlantic	1955	0	647	1,319	881	413	198	0	3,458	
	1956	0	579	1,195	1,228	557	302	0	3,861	
	1957	0	590	1,435	1,332	689	340	0	4,386	
	1958	0	184	519	654	432	280	0	2,069	
	1959	0	350	1,085	996	764	265	0	3,460	
	1960	0	611	800	1,092	382	236	0	3,121	
	1961	0	584	750	1,259	403	269	0	3,265	
	1962	0	624	344	419	409	347	0	2,143	
	1963	0	453	422	624	301	217	0	2,017	
	1964	0	94	233	183	125	30	0	665	
	1965	0	171	190	198	150	0	0	709	
	1966	0	9	19	125	70	0	0	223	

abundance, declined drastically in the Middle Atlantic area in 1963, the mean tons per set also declined. But in the North Atlantic area, where the catch per vessel week also dropped sharply in 1963, the mean tons per set did not drop until 1965. Where the decline in the catch per vessel week was not so severe, no changes in the catch per set were noted. Perhaps population density must reach a rather low level before it can cause a significant decrease in school size. A factor which may contribute to an ostensible decrease in school size is selectivity by vessel captains, who have a tendency to pass by the smaller schools when fish are abundant. When fish are scarce, captains are less discriminate.

VARIATION IN ABUNDANCE

Atlantic menhaden are pelagic, but they rarely range far from shore. Most are caught within 20 miles of the coast. People have speculated that a large population, unavailable to the fishery,

TABLE 11.—Mean number of purse-seine sets per vessel day in the Atlantic menhaden fishery, by year, month, and area. Raw means are weighted, column means are unweighted.

Area	Year	Month							Mean	North Carolina fall fishery
		Apr.-May	June	July	Aug	Sept	Oct.	Nov.-Dec.		
South Atlantic and North Carolina fall fishery	1955	--	2 42	2 54	2 04	--	--	0	2 40	2 14
	1956	2 00	2 64	1.36	2.00	3 00	--	0	2.07	2.49
	1957	1 65	2.44	--	2 30	1 67	1 20	0	1 97	2.79
	1958	3 12	2 75	2 00	1.54	2.36	--	0	2.47	2.35
	1959	2 30	2 67	2.43	2.46	2 60	--	0	2 50	2.42
	1960	2 36	2 45	2.82	2.97	--	--	0	2 70	2.76
	1961	2.69	1 79	2.88	--	--	--	0	2.20	2.11
	1962	2 65	2.69	3.24	2.85	3.60	2 88	0	2 88	2 39
	1963	2 32	2 21	2.50	3 00	3 40	3 50	0	2.41	2 62
	1964	2 25	2 43	2.40	2.35	2 63	--	0	2 41	2.60
	1965	1 71	2 39	2.23	1 90	2.32	3 33	0	2.17	2.78
	1966	1 72	2 24	1 95	2.51	2 06	2 00	0	2.09	2.76
	Mean	2 25	2 43	2.40	2.36	2 62	2 58	0	2 36	2.52
Chesapeake Bay	1955	--	--	--	--	--	--	--	--	
	1956	0	3.70	3.49	3.98	3 82	3 33	0	3.72	
	1957	0	4 43	4 81	4.72	4 89	3 10	0	4.56	
	1958	0	3 45	3 89	4 08	5.08	2 85	0	3 99	
	1959	0	4 13	4 10	5 32	5.27	3 73	0	4 50	
	1960	0	3 82	4 19	3.95	4 34	3 72	0	4 00	
	1961	0	4 57	4 00	3.68	3 65	3 48	0	4.01	
	1962	0	2 93	3 15	3.30	2 76	2 71	0	2 98	
	1963	0	3.75	2.34	2.82	3.20	3.69	0	3 25	
	1964	0	3 21	3.00	3 75	3 63	3.09	4 03	3 38	
	1965	0	3 22	2 69	3 08	2.88	3 31	4 07	3.06	
	1966	0	2 67	2 92	3.53	2 79	3 05	3.07	3.03	
	Mean	0	3 62	3.51	3.84	3 85	3 28	3.72	3.68	
Middle Atlantic	1955	0	3 38	4 15	3 83	3.38	2 68	0	3.61	
	1956	0	3 79	4.17	4.40	3.51	2 41	0	3.87	
	1957	0	3 50	4 50	4 67	4.03	3 21	0	4 07	
	1958	0	2 63	3.19	3 41	3 89	2 53	0	3.33	
	1959	0	3 30	3.93	4.16	3 45	2 65	0	3.68	
	1960	0	4 22	4 68	4 51	4 00	2 89	0	4 26	
	1961	0	3.24	4.04	4 48	3 68	2 96	0	3 82	
	1962	0	3 84	3 03	4 21	3.15	2 12	0	3.45	
	1963	0	3 15	3 13	3 30	3 20	1 96	0	3.07	
	1964	0	2.41	3.19	3 56	3 01	--	0	2 98	
	1965	0	3 35	3 28	4 01	2 78	3 62	0	3 40	
	1966	0	2 79	--	3.95	2 75	--	0	3.08	
	Mean	0	3.30	3 75	4.04	3 40	2.70	0	3 55	
North Atlantic	1955	0	2 49	3.23	2 45	1 98	2 28	0	2 48	
	1956	0	2 63	2.89	3 41	2 53	2 25	0	2 84	
	1957	0	3 80	3 55	2 96	2 89	2 97	0	3.28	
	1958	0	2 13	2.93	2 83	2.66	2 37	0	2.70	
	1959	0	2 14	2 53	3 46	2 60	1 97	0	2.73	
	1960	0	2.70	2 74	3.64	2 07	2 11	0	2.79	
	1961	0	2.75	3.24	3 77	2.54	2 60	0	3 12	
	1962	0	3 06	2.77	3.56	2 73	2 61	0	2.96	
	1963	0	2 54	3 42	2.96	2 61	1 67	0	2.75	
	1964	0	1 87	3.26	2.48	2 13	1 92	0	2 58	
	1965	0	2 87	2 96	2 54	2 39	--	0	2.71	
	1966	0	1 40	2.78	5 00	--	--	0	2.24	
	Mean	0	2 53	3.03	3.26	2 48	2 28	0	2 77	

may occur far offshore. There is no available evidence to support this view.

There is evidence, however, that the entire population is fished. Since 1945 Atlantic menhaden have been exploited from northern Florida to the Gulf of Maine, an area constituting nearly their entire range. With the advent of airplanes that could search larger areas and vessels that could range up to a hundred miles from port, no areas have been unsearched or unfished, except where prohibited by local restrictions.

Under these conditions changes in the catch per unit of effort are assumed to reflect changes in actual, rather than apparent, abundance. Even though the figures have been influenced by changes in vessel efficiency, they are sensitive

TABLE 12.—Mean catch per purse-seine set of Atlantic menhaden, in metric tons, by year, month, and area. Row means are weighted, column means are unweighted.

Area	Year	Month						Mean	North Carolina fall fishery
		Apr.-May	June	July	Aug.	Sept	Oct.		
South Atlantic and North Carolina fall fishery	1955	16.4	11.9	11.3	7.4	15.9	16.9	12.6	44.4
	1956	19.4	21.9	16.1	12.7	13.6	13.6	17.7	31.2
	1957	10.7	17.3	14.5	10.0	12.2	11.1	13.1	35.6
	1958	10.4	15.3	21.4	8.4	9.1	21.4	13.5	29.8
	1959	16.8	16.9	11.2	10.0	9.5	9.7	13.2	44.8
	1960	13.2	12.0	13.8	16.5	13.0	48.7	16.0	44.2
	1961	22.6	24.9	15.4	14.6	17.9	15.3	18.6	53.3
	1962	14.2	15.3	12.4	16.4	9.3	13.4	13.2	45.5
	1963	16.4	18.5	18.9	13.4	7.5	3.2	14.0	28.3
	1964	25.6	22.5	23.6	15.5	3.6	--	20.0	27.6
	1965	17.4	25.7	17.2	15.0	10.7	10.3	17.2	28.8
	1966	20.2	15.3	19.2	18.4	21.1	12.3	18.2	44.7
	Mean	17.0	18.1	16.2	13.2	12.0	16.0	15.6	38.2
Chesapeake Bay	1955	--	--	--	--	--	--	--	
	1956	--	12.6	14.3	10.8	12.9	9.1	12.2	
	1957	--	11.8	11.9	9.6	13.1	11.1	11.4	
	1958	--	11.9	20.7	18.9	16.0	8.8	16.4	
	1959	--	15.3	13.2	14.8	16.0	18.1	15.2	
	1960	--	21.8	14.2	14.1	11.2	14.0	15.1	
	1961	--	16.1	20.0	12.2	16.9	11.1	16.1	
	1962	--	29.1	15.2	16.9	13.2	23.6	18.5	
	1963	--	12.9	16.2	15.8	12.4	11.9	13.9	
	1964	--	14.2	13.4	13.3	9.3	15.3	13.2	
	1965	--	15.9	15.7	13.6	11.9	13.0	13.9	
	1966	--	11.9	11.7	8.4	10.5	13.8	12.6	
	Mean	--	15.8	15.1	13.5	13.1	13.6	14.4	
Middle Atlantic	1955	--	23.0	23.8	21.3	23.3	37.4	24.0	
	1956	--	29.1	25.7	25.6	22.2	30.7	26.4	
	1957	--	16.5	18.4	15.5	14.6	30.0	18.0	
	1958	--	18.1	19.1	24.0	22.7	15.0	21.5	
	1959	--	22.8	18.1	18.8	16.6	26.1	19.6	
	1960	--	15.6	17.3	16.2	15.3	21.7	16.8	
	1961	--	19.4	22.8	15.2	12.7	15.5	17.7	
	1962	--	22.6	16.8	8.3	15.1	48.7	19.8	
	1963	--	17.2	9.5	6.3	10.1	16.5	12.7	
	1964	--	10.7	10.8	13.3	11.2	11.0	11.3	
	1965	--	13.0	10.2	14.2	17.1	27.2	13.7	
	1966	--	7.0	4.7	10.3	16.3	--	8.0	
	Mean	--	18.0	16.4	15.8	16.4	25.4	17.4	
North Atlantic	1955	--	12.9	21.2	31.2	26.9	41.6	24.0	
	1956	--	23.2	24.4	25.1	26.8	33.9	25.5	
	1957	--	17.7	15.4	18.1	24.1	29.6	19.1	
	1958	--	14.4	10.5	16.9	18.6	16.7	15.4	
	1959	--	19.5	18.9	18.9	17.1	26.0	19.0	
	1960	--	24.3	19.2	18.8	19.8	34.7	21.2	
	1961	--	14.3	19.5	18.9	16.7	24.9	18.0	
	1962	--	22.9	21.3	17.6	22.8	44.7	25.1	
	1963	--	18.6	9.4	20.1	19.7	19.9	17.4	
	1964	--	22.8	30.2	19.3	23.8	34.3	25.1	
	1965	--	13.2	14.3	20.8	18.5	--	16.7	
	1966	--	4.4	4.3	8.5	9.5	--	8.3	
	Mean	--	17.3	17.4	19.5	20.3	30.6	19.6	

enough to reflect real differences in population abundance.

Variations in year-class strength have contributed to fluctuations in population abundance. Estimates of year-class strength prior to 1952 have been based on catch-per-unit-of effort figures and since 1952 on catch samples. From 1950 to 1958 there were four exceptionally large year classes, the largest occurring in 1958 (June and Reintjes, 1959; Nicholson and Higham, 1964). Most of the year classes after 1958 have been smaller than any of the year classes prior to 1958. This series of small year classes occurred simultaneously with the general decline in abundance, which began about 1956 and became more noticeable in 1963, after the large

1958 year class had nearly passed from the fishery.

The greatest decline in abundance has been in the Middle and North Atlantic areas, where older fish constitute the bulk of the catches. Fish pumps and airplane spotters, two of the improvements having the most impact on fishing effectiveness, increased sharply in both areas after 1949. Yet the catch per vessel week, after reaching a peak in about 1952, declined thereafter in both areas, despite other fishing improvements added in the middle 1950's. In the North Atlantic, the catch per vessel week, except for 1952 and 1953, was no greater from 1950 through 1962 than it had been from 1941 to 1950. In the Middle Atlantic, the catch per vessel week, although being substantially greater from 1952 to 1962 than it had been up until 1951, began a steady decline in 1957, and from 1963 to 1966 was much lower than in the years prior to 1950. From these data one may conclude that the abundance of menhaden in these two areas was no greater from 1950 to 1962, and considerably less after 1962, than it had been prior to 1950.

The decline in abundance in Chesapeake Bay, where 1- and 2-year-old fish compose most of the catches, has not been as great as in the North and Middle Atlantic. The catch per vessel week was substantially greater from 1954 to 1962 than it was prior to 1954 or after 1962. Since the major improvements in fishing methods came a few years later than in the Middle and North Atlantic, the higher catches per vessel week after 1953 probably reflect an increase in fishing efficiency, although they could reflect an increase in menhaden abundance. The decrease after 1962 probably resulted from a true decrease in menhaden abundance.

Abundance in the South Atlantic, where age-1 fish compose most of the catch, appears to have remained unchanged. The catches per vessel week varied widely, but showed no trend. In this area the fisheries at the three ports are small, geographically distinct, and dependent on relatively small numbers of fish, principally of one age group, that are dispersed over a large area. If the carrying capacity is less in the South Atlantic than in other areas, the abundance of fish in the area is less likely to reflect changes in the total Atlantic menhaden population than is the abundance of fish in areas of high density and high carrying capacity, such as Chesapeake Bay.

In the North Carolina fall fishery menhaden nearly always will appear to be abundant, because they are concentrated in a small area for a short period of time and are easy to catch. But since weather is more variable than in other areas, it influences the catch per vessel week more than it does elsewhere. The wide fluctuations in the catch per vessel week, therefore, do not necessarily reflect variations in abundance.

The relation between the decline in abundance and the high levels of fishing effort can be understood only if the spawning age, the age and size distribution, and the seasonal movements of the fish are considered. Atlantic menhaden spawn after they have completed three growing seasons (Higham and Nicholson, 1964), and rarely survive past seven growing seasons. Their age and size distribution and seasonal movements have been described by June and Nicholson (1964) and Nicholson,[2] and are briefly summarized here.

During the fishing season from about May to October, the population from Florida to Chesapeake Bay is composed primarily of age-1 and age-2 fish. Although the proportion of each age group varies with the strength of individual year classes, age-1 fish are usually more abundant, particularly south of Cape Hatteras. From the mouth of Chesapeake Bay to Long Island, age-2 fish gradually replace age-1 fish as the dominant age group. Age-3 fish, dominant in Long Island and Nantucket Sounds, become less abundant north of Cape Cod, where age-4 to age-7 fish predominate. A southward movement begins among fish at the northern end of the range in late summer and extends to all fish north of Cape Hatteras by early November. By mid-January nearly all menhaden have moved into the offshore area between Cape Lookout and northern Florida. In late winter these fish begin a northward movement.

[2] Nicholson, William R. Movements of Atlantic menhaden as inferred from changes in age and size distribution. (Unpublished manuscript.)

As older fish decreased in abundance, fisheries dependent on them declined. No menhaden were landed after 1958 at Portland, after 1962 at Gloucester, or after 1963 at Point Judith. After the 1958 year class ceased to contribute large numbers to the catch, the Amagansett, Port Monmouth, and Tuckerton catches dropped sharply. As catches declined and plants closed or reduced fishing, effort also dropped. By 1968 only 136 vessel weeks were expended in the North and Middle Atlantic, as compared with 1,265 in 1962.

Effort in areas where age-1 and -2 fish were predominant continued to be high. In 1968, 846 vessel weeks were expended in the South Atlantic and Chesapeake Bay, as compared with 909 in 1962.

Changes in the catch and the catch per vessel week suggest that the decline in numbers of fish older than age 2 was much greater than the decline in numbers of fish younger than age 3.

If recruitment is dependent on spawning population size, and spawning population size is dependent on the escapement of prespawning age fish, the total yield will be limited by the amount of escapement. Schaaf and Huntsman[3] have shown that with present levels of fishing effort, the spawning stock of Atlantic menhaden is inadequate for recovery of the population.

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ABUNDANCE AND DISTRIBUTION OF YOUNG ATLANTIC MENHADEN, *Brevoortia tyrannus*, IN THE WHITE OAK RIVER ESTUARY, NORTH CAROLINA

E. Peter H. Wilkens[1] and Robert M. Lewis[2]

ABSTRACT

The effect of salinity, temperature, tide, turbidity, and illumination on the distribution of larval, prejuvenile, and juvenile menhaden in an estuary was investigated. Most menhaden larvae entered the estuary in March after the water had warmed to about 10° C, and moved upstream to the low-salinity-freshwater zone where they transformed into juveniles. More larvae were caught in the lower estuary on flood tide. After transformation to juveniles they were caught in schools throughout the estuary. Turbidity and illumination did not affect the distribution of menhaden, but illumination affected catchability, since more menhaden were collected during night tows.

In this study we investigated the effects of temperature, salinity, and light on the distribution and abundance of larval, prejuvenile, and juvenile menhaden in a single estuary, the White Oak River in North Carolina. As the strength of individual year classes of Atlantic menhaden fluctuates widely, we were interested in determining what effect these environmental factors had on menhaden during their first year in an estuary. This information could be helpful in assessing and predicting the strength of individual year classes. Estimates of the strength of individual year classes before they enter the fishery would be valuable to the commercial fishery. A single estuary was selected so that a better understanding of these variables could be achieved before being applied to a coastwise fishery.

Atlantic menhaden, found along the eastern coasts of the United States and Canada from Nova Scotia to southern Florida (Hildebrand, 1964; Reintjes, 1964), spawn in almost every month in some part of their range (Higham and Nicholson, 1964). Menhaden are spawned in the ocean and enter an estuary as larvae. Once strong enough to swim against the tidal currents, they move upstream towards fresh water (Lewis and Mann, 1971) where they transform into juveniles. They remain in the estuary for their first growing season, gradually moving downstream in the summer and reaching the lower estuary or open sea by autumn (June and Chamberlin, 1959; Massmann, Ladd, and McCutcheon, 1954).

STUDY AREA, SAMPLING LOCATIONS, PROCEDURES, AND NETS

The White Oak River estuary is a bar-built estuary (Pritchard, 1967) which drains forest lands in the upstream part and salt marshes in the downstream part (North Carolina State Board of Health, 1954). The Intracoastal Waterway crosses the mouth of the estuary near Bogue Inlet.

We selected this estuary because: (1) We knew from preliminary sampling that larval menhaden entered the lower section from the ocean in the winter and early spring and that juveniles occurred in the upper section in the summer; (2) its small size (28 sq km) permitted ample sampling coverage; and (3) its proximity to the Beaufort laboratory made frequent sampling easy.

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We assumed that few or no larvae moved from other estuaries into the White Oak River estuary.

We collected larval, prejuvenile, and juvenile menhaden at 14 sampling locations extending 34 km upstream from Bogue Inlet (Figure 1). The distance between stations varied from 2 to 5 km and averaged about 3 km. The type of gear we used, frequency of sampling, and date of sampling are listed in Table 1.

TABLE 1.—The White Oak River estuary sampling schedule.

Sampling location	Sampling frequency	Gear	Sampling period
Swansboro Bridge (Station 2)	2-3 days/week	Channel net	Nov. 1967-Apr. 1968 Nov. 1968-Apr. 1969
Upstream stations 6-12	Monthly	Surface trawl	July-Sept. 1968
Upstream stations 6-12	Weekly	Channel net	Feb.-Apr. 1969[1]
Upstream stations 6-12	Biweekly	Channel net	May 1969
Upstream stations 6-12	Monthly	Channel net	June-Sept. 1969[2]

[1] Stations 1, 3, 4, and 5 were sampled irregularly in this period.
[2] A few samples were taken upstream with the surface trawl in this period.

We sampled larvae entering the estuary at the Swansboro Bridge (station 2) 2 or 3 days per week from November through April in 1967-68 and 1968-69. During July, August, and September 1968, we sampled at several of the upstream stations. Starting in February 1969 we collected menhaden throughout the river and estuary, although our efforts were concentrated on the upstream section (stations 6 through 12). In March and April we visited these stations every week; in May, every 2 weeks; and from June to September, every month.

During the 1967-68 and 1968-69 seasons, we sampled for larvae with a channel net described by Lewis, Hettler, Wilkens, and Johnson (1970). The net, with a 1 by 3 m opening, had a tail bag constructed of 0.5-mm mesh. At Swansboro we attached the net to the bridge and made four to six 30-min sets per day. At the other locations, we towed the net between two 16-ft aluminum boats and made a 15-min set when the larval and prejuvenile menhaden were scarce and a 5-min set when they were abundant.

During July, August, and September 1968, we sampled juveniles with a 6.1-m surface trawl

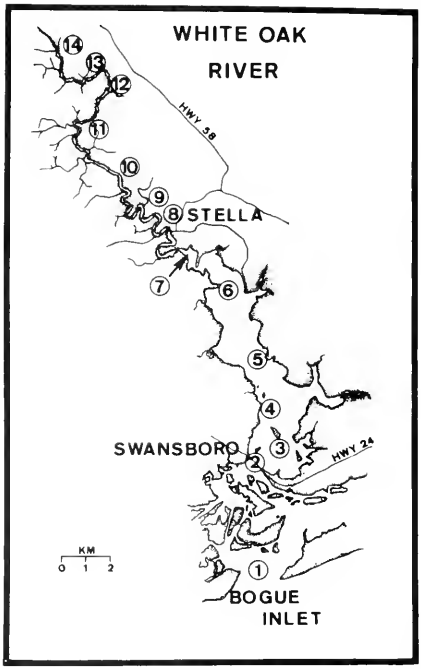

FIGURE 1.—Sampling locations for young menhaden on the White Oak River estuary, N.C.

having a 6.2-mm bar mesh. We towed it in the same manner as the channel net. During the corresponding months in 1969, most of the collections were made with the channel net.

In all the collections, the number of menhaden caught was expressed as an index (I = number of young menhaden per 100 m^3 water strained).

We recorded surface temperature and salinity at the start and end of each set at the bridge and at the end of each tow at the other sites. We measured the amount of water strained by the net during each set with a flowmeter. Dissolved oxygen and turbidity readings were taken only in July, August, and September 1968.

TEMPORAL DISTRIBUTION

Larval menhaden entered the White Oak River from November until early May. Two peaks of abundance occurred each year, one in November and December and the second and major peak in February and March (Figure 2).

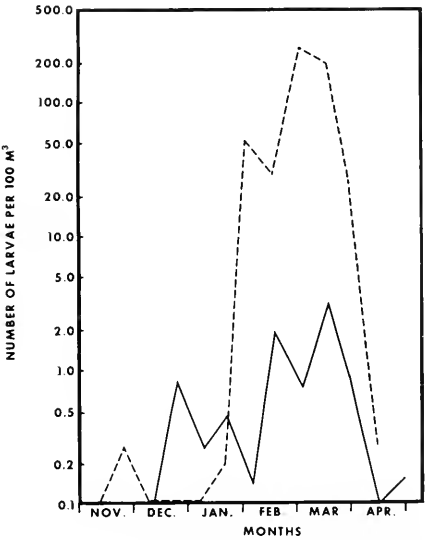

FIGURE 2.—The mean biweekly abundance indexes at Swansboro Bridge in 1967-68 (solid line) and 1968-69 (dashed line).

The entrance of large numbers of larvae in February and March probably resulted from the migratory schools of menhaden that spawned off the North Carolina coast during the winter. Higham and Nicholson (1964) found that during November and December most of the females in the landings were nearly ripe. Reintjes (1969) reported finding hundreds of thousands of developing menhaden eggs off the North Carolina coast in December 1966.

Those larvae that entered the estuary during November and December were probably the progeny of fish that either had inhabited North Carolina waters in the summer or had moved into North Carolina waters from other areas in early autumn.

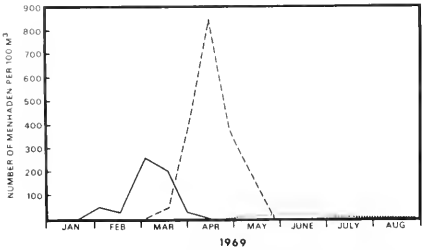

FIGURE 3.—The relative abundance of larval (solid line), prejuvenile (dashed line), and juvenile menhaden (dotted line) in the White Oak River estuary. (The larval indexes are from the bridge, and the prejuvenile and juvenile indexes are from the upstream zone where the fresh and salt water mix. Larval and prejuvenile indexes are biweekly means, but the juvenile index is a monthly mean plotted on the day the sample was obtained.)

Prejuvenile menhaden were first caught in March and became abundant in late March and April (Figure 3). After the peak in April the number of prejuveniles decreased, and by the beginning of May most had transformed into juveniles.

Juvenile menhaden, collected in relatively low numbers from May until September, occurred with maximum abundance in late May and June. As young menhaden got above 15 mm fork length during the summer of 1968, we caught fewer fish during the day. To determine if illumination was a factor that resulted in greater net avoidance by juvenile menhaden, we scheduled a series of day-night sampling trips. We collected larger samples of menhaden during nighttime tows, which indicated that some fish were able to avoid our net during the day. Fish from day and night tows were similar in length. In addition, we caught more menhaden on overcast or moonless nights than on clear, moonlight nights. As a result of our findings in 1968, we sampled for juveniles in 1969 only at night in order to increase our sampling efficiency.

EFFECT OF SALINITY, TEMPERATURE, AND TIDE ON THE DISTRIBUTION OF YOUNG MENHADEN

SALINITY

Larval menhaden, after entering the lower estuary, move upstream into lower salinities to metamorphose. They seek the zone of the river from 1 ‰ salinity to fresh water. This zone extends a short distance upstream from the interface between fresh and salt water. Larval and prejuvenile menhaden were most abundant in this zone where metamorphosis occurs (Figure 4). They apparently range into fresh water for only a short distance since they were absent or present only in small numbers in our samples farther upstream.

After the menhaden have transformed from prejuveniles to juveniles, they appear to seek higher salinity water. In late May when most of the prejuveniles had metamorphosed, we found juveniles in low-salinity water. As the season progressed the juveniles were present in the low-salinity water upstream, but they tended to be more abundant in water downstream. Schools of juvenile menhaden generally moved out of the estuary in the fall.

Both the position and length of the upstream zone where young menhaden concentrated are influenced by tidal excursion, rainfall, and wind direction and strength. A northeast wind causes unusually high tides and pushes salt water farther upstream than during normal flooding and ebbing tides.

We do not understand why low-salinity water is important to young menhaden, but one explanation is that they cannot metamorphose properly in either fresh or high-salinity water. When larvae were held in salinities ranging from 15 to 40 ‰, about one-third of the fish in each salinity group developed abnormalities of the spine (Lewis, 1966). Juveniles may also congregate in low-salinity areas at times because food may be more abundant.

In some estuaries turbidity may vary with salinity. We measured the turbidity at the Swansboro site in March, April, and May 1968 and at our upstream sites during the summer of 1968 to determine if it affected the number of young menhaden caught by our net, and to see if there was any relation between salinity and turbidity. At Swansboro, where the water remained relatively clear (light transmittance ranged from 89 to 96%), no correlation existed between turbidity and the catch of larvae. At our upstream tow areas, where the water also was clear, light transmittance ranged from 68 to 95%. We also found no relation between turbidity and catch of young menhaden. We concluded therefore that turbidity was too low to affect catchability in the White Oak River. As there were no marked differences between up- and downstream turbidities, we concluded also that there was no relation between salinity and turbidity.

TEMPERATURE

Larval menhaden are sensitive to low temperatures, particularly if the salinities are high or low. They have the best chance for survival in an estuary if the temperature remains above 4° C and the salinity ranges between 10 and 20 ‰ (Lewis, 1966). Below 4° C they survive for only a short time. Lewis (1965) determined that the number of hours to 50% mortality at 2.0° C varied from 3.2 to 38.5 hr depending on the acclimation temperature.

We compared the temperatures in the estuary during the 2-year study with temperature tolerances of larval menhaden determined in the laboratory by Lewis (1965, 1966). The water temperature in the White Oak River went below 4° C for several days in January and December 1968 and January 1969. Except for two sampling days in 1968 and one in 1969, the water temperature did not get over 10° C from the beginning of January to mid-March; it stayed at 2° C for 2 days during this period in 1968, and 1 day in 1969. We caught few menhaden larvae during the periods of low water temperature.

Most larvae that enter the estuary before the lethal cold water temperatures in the winter probably do not survive, while those that enter during the late winter and early spring probably remain in the downstream area because of the colder water upstream. As the water warms in the spring to above 10° C they move towards

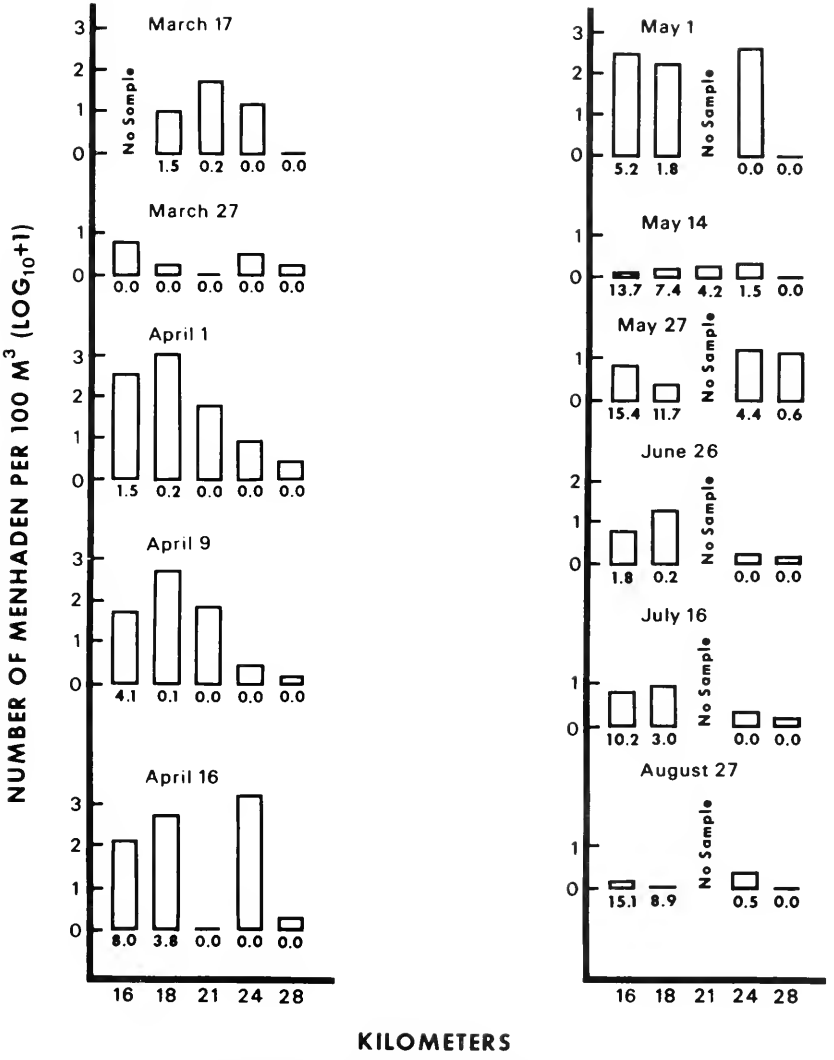

FIGURE 4.—The distribution of menhaden by date, kilometer, and salinity in the White Oak River, March-August 1969. (Number below each bar is salinity at station.)

the low-salinity freshwater zone. Those that enter later in the season, after the water has warmed, move upstream in a shorter time. Once menhaden had moved upstream, salinity and food supply probably affected their distribution more than temperature.

Since dead menhaden larvae do not float, we probably would not notice if kills occurred when the temperature dropped below 4° C. However, we did observe many dead young and adult bay anchovy (*Anchoa mitchilli*) and pinfish (*Lagodon rhomboides*) floating on the surface on January 10 and 12, 1968, when the water temperature was 2° C. Some of the floating fish revived when placed in warmer water, but most did not. We assume that many of the species present in the estuary either died from or were subject to cold stress. Thus any cold weather that occurs during the time larval fish are present in the estuary can have an important effect on the number of individuals surviving in the population.

Although high water temperature does kill juvenile menhaden, it did not appear to cause any mortality in the White Oak River estuary. In laboratory tests, juvenile menhaden died in water temperatures above 33° C (Lewis and Hettler, 1968). In the White Oak River the temperature remained below 33° C except for a short period when it rose to 34.1° C.

TIDE

Velocity of water current affects the abundance and distribution of fish larvae in an estuary. Bishai (1959) found that herring larvae (6-8 mm total length) maintained themselves in a current of 0.58 to 1.03 cm sec and that at higher velocities they drifted with the current but at a rate less than the current.

During our sampling in the field we noticed that larval menhaden also held their positions only in weak currents. The menhaden larvae collected at the Swansboro bridge were larger (10-30 mm total length) than the herring tested by Bishai and seemed able to maintain their position at velocities less than 10 cm sec. Above this velocity they were carried by the current. One would expect, therefore, to obtain large larval indexes during peak tidal flows at midflood and at early ebb. Larval indexes during these periods varied considerably, but in general were larger than indexes during late ebb and early flood tide stages (Table 2). The variability in abundance indexes arises, in part, from day-to-day changes in menhaden distribution in the lower estuary during the 2-month period. A 24-hr study in March 1968 at Beaufort, N.C., showed that larval abundance varied with the tide, current, and time of day (Lewis and Wilkens, 1971).

TABLE 2.—Number of menhaden larvae per 100 m^3 of water at hourly flood and ebb-tide stages, Swansboro, N.C., February-March 1969.

Stage	Hour	Mean velocity	Abundance index	Range	Number of collections
		m/sec			
Flood	1	0.10	33.6	17.2- 45.2	3
	2	0.17	216.0	16.0- 591.4	4
	3	0.15	94.9	0.8- 578.4	8
	4	0.18	74.0	4.0- 338.8	7
	5	0.15	894.9	1.5-3,582.0	7
Ebb[1]	1	0.22	65.6	20.6- 110.6	2
	2	0.27	168.0	25.6- 365.4	3
	3	0.23	43.1	7.2- 121.1	5
	4	0.21	26.4	0.4- 90.5	6
	5	0.19	17.8	2.2- 82.9	7
	6	0.16	6.5	1.9- 13.0	8
	7	0.11	8.7	1.4- 14.4	7

[1] The ebb-tide stage generally lasted about 2 hr longer than flood.

Tides affect the movement of larvae in and out of an estuary as well as within an estuary. Flooding currents carry larval menhaden into the estuary where, before heading upstream, they move back and forth with the changing tides. At the Swansboro bridge station more larvae were caught on flood tide than on ebb. During February and March 1969, larval indexes greater than 10 occurred in 81% of the sets made on flood tide but only in 51% of the sets on ebb tide. June and Chamberlin (1959) reported similar results at Indian River, Del. Some of the larger catches of the season occurred on late flood. The early hours of ebb tide had higher larval indexes than late ebb. As more larvae enter than leave, the number of larvae in the estuary reaches a maximum by midspring.

Tidal stage and current velocity affected the catchability of larvae. In the lower estuary these forces either concentrated the larvae in one location or spread them over a large area. In the upper estuary the location and width of the low-

salinity-freshwater zone, which was influenced by the tide, affected the distribution of larvae and prejuveniles.

SUMMARY

Larval menhaden were present in the White Oak River estuary from November to May but were most abundant in February and March. Prejuveniles were abundant in late March and April, and by the beginning of May most had transformed into juveniles. Our largest catches of juveniles occurred in May.

Larvae progress upstream to the zone where the salt and fresh water mix (0-1 ‰ salinity). Large catches of prejuvenile and larval menhaden occurred within this zone. We did not find juvenile menhaden in the zone until the end of May when most prejuveniles had transformed to juveniles.

Laboratory tests from other studies showed that menhaden died when the water temperature fell below 4° C and rose above 33° C. Even though young menhaden encountered both extremes of water temperature in the White Oak River, we saw no evidence of any deaths.

Catches of larval menhaden at the Swansboro bridge were more abundant on flood tide than ebb. The early hours of ebb tide had higher larval indexes than late ebb.

Illumination affected the catches of juvenile menhaden at our upstream stations as we caught more menhaden on overcast or moonless nights than on clear, moonlight nights or during the daylight hours.

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Isistius brasiliensis, A SQUALOID SHARK, THE PROBABLE CAUSE OF CRATER WOUNDS ON FISHES AND CETACEANS

EVERET C. JONES[1]

ABSTRACT

Evidence is presented that bites inflicted by the small squaloid shark, *Isistius brasiliensis* (Quoy and Gaimard), are the causes of crater wounds, crescentic wounds, and related scars on large pelagic fishes and cetaceans. This evidence consists of a crescentic "wound" experimentally produced on the side of a dead fish by a living *Isistius*; specialized morphology of the shark's basihyoid cartilage and coracohyoideus muscles, lips, labial cartilages, and spiracles, that, together, enable the shark to form an oral vacuum on a smooth surface; an experiment in which a living *Isistius* formed such a vacuum; specialized morphology and arrangement of the mandibular teeth; close agreement between the range of reported wound widths and the estimated range of bite widths of *Isistius;* agreement between the geographical ranges of *Isistius* and those fishes and cetaceans which bear crater wounds; and, finally, the presence in *Isistius* stomachs of hemispheroidal plugs of fish flesh. Speculation on the circumstances that may enable a small, slow shark to make contact with large, swift fishes and cetaceans is included. *Isistius* apparently qualifies as a temporary parasite.

Probably the earliest account of the existence of small, round or oval, scooped-out wounds on the sides of large pelagic fishes is contained in an ancient legend of Samoa (A. Utu, personal communication), which states that atu (skipjack tuna, *Euthynnus pelamis* (Linnaeus)) entered Palauli Bay, and, upon approaching the beach, left small round pieces of their flesh as gifts to Tautunu, chief of that community. Evidence of this sacrifice was found by the people who caught the atu and observed fresh, round wounds on their sides.

This legend provides one of many explanations that have been advanced regarding the causes of such wounds on large pelagic fishes as well as on whales and porpoises. This paper presents evidence that many crater wounds, crescentic wounds, and the resulting scars on pelagic fishes (Figure 1), and open pit wounds and resulting scars on cetaceans are the results of bites inflicted by the small squaloid shark, *Isistius brasiliensis* (Quoy and Gaimard). (A second species, *Isistius plutodus*, was described by Garrick and Springer (1964) from the Gulf of Mexico. Although nothing is known of the behavior of this species, which is based on one specimen, it is probable that its feeding habits are similar to those of *I. brasiliensis*.)

Such wounds on fishes have been reported by Nemoto (1955), Iversen (1959), Guitart M. (1964), Klawe (1966), Bane (1969), and Machado Cruz (1969). The literature on open pit wounds and related scars on cetaceans is much more extensive, apparently beginning with the work of Collett (1886). Mackintosh and Wheeler (1929) and van Utrecht (1959) presented thorough discussions of these wounds and probable causes, and summarized the previous literature. Nemoto (1955) noted that some of the wounds observed on whales are similar to and probably have a common origin with those on fishes. He further stated that a cause other than lamprey attacks, the most commonly advocated agent, must be found to account for crescent-shaped scars and open pit wounds on cetaceans and fishes.

It was not always possible for me to determine whether published photographs and descriptions were of wounds and scars of the types which I attribute to *Isistius* bites. I have never seen wounds known to have been produced by lampreys and therefore cannot comment with any

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FIGURE 1.—Crater wounds on a large dolphin from the central Pacific.

authority on them. I believe, however, that Pike (1951) unknowingly but accurately described the differences between lamprey bites and *Isistius* bites on whales when he wrote: "The lampreys seem to leave two distinct types of wounds . . . . The first consists of a circular area in which the epidermis is completely abraded by the teeth of the sucking disc. In the center of this is a hole through the skin caused by the rasping tongue. In the other type the lamprey apparently rasps away the skin over the entire area with the result that there is a circular sore right down to the blubber and no periphery of skin which has been damaged but not eaten away." The second type and some of the wounds and scars illustrated by Pike are, I believe, attributable to *Isistius* bites.

Crater wounds have been reported in the literature cited on skipjack tuna; yellowfin tuna, *Thunnus albacares* (Bonnaterre); dolphin, *Coryphaena hippurus* Linnaeus; opah, *Lampris regius* (Bonnaterre); and swordfish, *Xiphias gladius* (Linnaeus) (Figure 2). In addition to these, I have seen crater wounds on albacore, *Thunnus alalunga* (Bonnaterre), and wahoo, *Acanthocybium solandri* (Cuvier), in the central Pacific. Biologists and fishermen in Hawaii have reported to me having seen them on kawakawa, *Euthynnus affinis* (Cantor); large jacks, *Caranx* sp.; rainbow runners, *Elagatis* sp.; and various species of marlins, *Istiophoridae*.

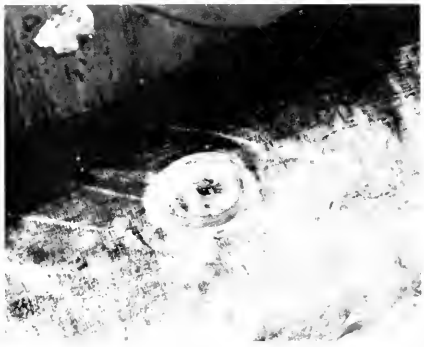

FIGURE 2.—A crater wound on the side of a swordfish caught in the Gulf of Mexico. (Photo by Martin Bartlett.)

The cetaceans upon which crater wounds, crescentic wounds, or resultant scars have been reported were listed by van Utrecht (1959). Included were beaked whales, sperm whales, various species of porpoises, and nearly all of the baleen whales (order Mysticeti) except the right whales (family Balenidae) which apparently do not migrate out of cold polar waters. In Hawaiian waters, wounds and scars (Figure 3) are commonly seen on porpoises of the genera *Tursiops* and *Stenella*, and have been observed on a beaked whale, *Ziphias* sp., stranded on Oahu.

Dr. Donald W. Strasburg, during discussions several years ago, planted the idea that *Isistius* might be the cause of crater wounds on fishes. He had found (Strasburg, 1963) that the man-

FIGURE 3.—Crescentic scar on a living porpoise, *Stenella roseiventris* Wagner, The Oceanic Institute, Makapuu, Hawaii.

dibular teeth of *Isistius* are shed as a unit and that the next set of replacement teeth are already erect and immediately functional when the previous set is shed. He wondered " . . . which aspects of *Isistius* biology require such a safeguard."

OBSERVATIONS

An opportunity to test the idea came in July 1969, during cruise 44 of the RV *Townsend Cromwell* of the NMFS, HAFRC (National Marine Fisheries Service, Hawaii Area Fishery Research Center). Nightly midwater trawl hauls were made along long 145° W between lat 14° N and 3° S in the central Pacific. The trawl catches contained occasional specimens of *Isistius*, some of which were alive but moribund when brought on board. I stated that this species of shark might be responsible for the crater wounds which we had observed on tunas a few hours earlier. This led John D. Fowler, Jr., research assistant aboard the *Townsend Cromwell*, to press the mouth of a moribund *Isistius* against the side of a dead fish, *Cubiceps* sp. The shark made a biting motion, producing a crescentic wound (Figure 4) that if completed would have been similar in size and shape to crater wounds observed on tunas. That shark could not be induced to repeat its performance, but Fowler's experiment led to further attempts to determine

FIGURE 4.—A crater "wound" produced on the surface of a nectarine by pushing the teeth of a dead *Isistius* into the fruit and then rotating the body of the shark around the point of attachment. In the center is a crescentic "wound" produced by a living *Isistius* when its mouth was pressed against the side of the dead fish.

whether adaptations in structure for specialized feeding behavior existed in *Isistius*.

The basihyoid cartilage or "tongue" of *Isistius* was large and thick in contrast to that structure in galeoid sharks. It was also unusually movable; with a pencil I was able to push the tongue caudad to a point just anterior to the first exterior gill opening (Figure 5). In that position the posterior margin of the tongue was elevated (dorsad) until the tongue was nearly vertical, fitting closely against the roof of the mouth, and

FIGURE 5.—A demonstration of the movability of the tongue of *Isistius*.

completely separating the mouth from the pharynx. Two ridges in the roof of the mouth corresponded to two grooves in the posterior margin of the tongue. This structural correspondence suggested that vertical positioning of the tongue was a normal occurrence. The movability of the tongue, as well as several other attributes described below, can only be demonstrated with specimens of *Isistius* that have not been fixed.

Observations of these anatomical characters led to the hypothesis that *Isistius* is capable of achieving a vacuum with its mouth on a smooth surface. Concurrently with the retracted, vertical positioning of the tongue, the lips protruded completely around the mouth. The fleshy lateral lips contained well-developed labial cartilages that caused them to be semirigid and, when protruded, to complete an ovoid of labial margins in a single plane (Figure 6). Such a structure in contact with a smooth surface enables the

FIGURE 6.—A demonstration of the coracohyoideus muscles of *Isistius*. Note also the protruded lips, the internal openings of the spiracles, and the mandibular teeth.

shark to form a vacuum when the spiracles are closed and the tongue then retracted.

In order to further determine if behavioral retraction of the tongue was probable, dissections were made of the ventral surface of the shark just posterior to the mandible. The paired coracohyoideus muscles that insert on the tongue were unusually large in *Isistius* (Figure

FIGURE 7.—Exposed coracohyoideus muscles of a large whitetip shark, central Pacific.

6). A comparative dissection of a large, fresh, whitetip shark, *Carcharhinus longimanus* (Poey) was made (Figure 7); the cross sectional areas in *Isistius* were estimated to be four times those of the same muscles in the whitetip shark, both relative to the total lengths of the sharks. Pulling caudad on the exposed coracohyoideus muscles of *Isistius* caused the tongue to retract to the nearly vertical position noted before; concurrently, the mouth gaped and the lips protruded. The tongue of the whitetip shark was not movable and pulling on the coracohyoideus muscles did not retract it.

Later I attempted to repeat Fowler's experiment by holding the mouth of a living *Isistius* against the side of a gempylid fish. In this case, the shark did not make a biting motion but, instead, the spiracles closed, the head flattened slightly, and an oral vacuum was formed by means of which I was able to lift the gempylid from the table with no other support.

It seemed that the remaining evidence needed to indict *Isistius* would be the presence of hemispheroidal plugs of fish flesh in their stomach contents. This evidence was found when two *Isistius* caught subsequently on the same cruise were found to contain single plugs of flesh of appropriate size and shape. One of these plugs was from a relatively large fish, judging from the thickness of the myomeres; the other was from a squid. During a later cruise in the same area, Reginald M. Gooding, fishery biologist,

FIGURE 8.—A hemispheroidal plug of unidentified fish flesh from the stomach of an *Isistius*, central Pacific.

found a very fresh plug of fish flesh complete with integument and some scales (Figure 8). The fishes from which these plugs were bitten have not been identified. In order to find such plugs, it is necessary to examine *Isistius* immediately after capture because digestion will usually continue for a time after the specimen is placed in formaldehyde or a freezer.

DISCUSSION

Further evidence relating *Isistius* to wounds on fishes may be present in a photograph (Figure 9) of a crescentic wound on the caudal fin of a swordfish (M. R. Bartlett, personal communication). The deep crescentic cut is opposed by an arc of small dents and scars. The size and arrangement of these correspond to the small, hooked upper teeth of *Isistius* (Figure 6). In addition, a series of white scratches extending from the small arc toward the crescentic cut appear to have been made by the upper teeth as the shark backed away from the incomplete bite. In this case, the shark's mandibular teeth must have encountered the large, bony ray in the edge of the caudal fin. The same fin bore an entire, cut-out wound near the posterior border (Figure 10).

The geographical distribution of records of *Isistius brasiliensis* (Strasburg, 1963; Parin, 1964) corresponds well with the general distributions of the species of fishes which bear crater wounds. Several authors (Mackintosh and

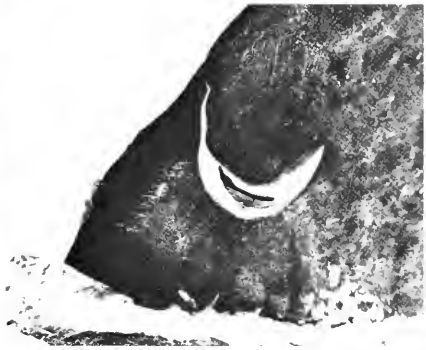

FIGURE 9.—A crescentic wound on the caudal fin of a swordfish caught in the Gulf of Mexico. Note the arc of small dents opposite the cut, and the scratches produced by the shark's upper teeth as it backed away from the incomplete wound. (Photo by Martin Bartlett.)

Wheeler, 1929; Pike, 1951; Nemoto, 1955; van Utrecht, 1959) have noted that fresh wounds were seen only, or more frequently, on cetaceans caught in the warmer waters of their migrations and that those caught more poleward bore only healed or partially healed scars. This was evidence, they stated, that the animal producing the wounds was an inhabitant of warm water.

Some wounds on cetaceans described in the literature were undoubtedly produced by lampreys (Pike, 1951). The majority of catch records of lampreys in both the Pacific and Atlantic, however, are near shore and in temperate or cold waters which fits poorly the distribution of fishes and whales bearing fresh crater wounds.

The largest crater wounds recorded (Mackintosh and Wheeler, 1929) were 4 or 5 cm by 7 cm. The smallest I have seen were 1.2 cm by 2 cm. The smaller diameters of these correspond well with the bite-widths I have estimated for *Isistius* at the extremes of the known range of 14 to 50 cm, total lengths (Strasburg, 1963).

All of the *Isistius* stomachs examined aboard the *Townsend Cromwell* contained squid beaks and pieces of squid pens. Strasburg (1963) also

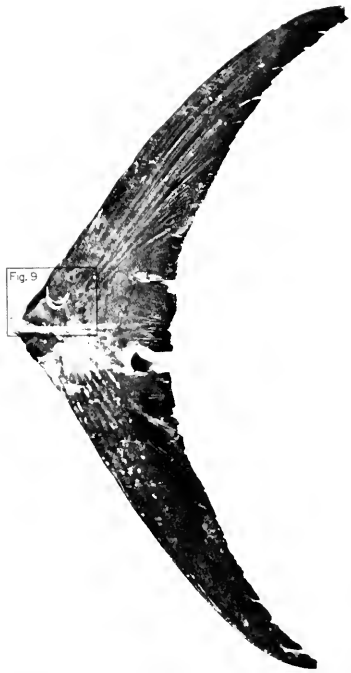

FIGURE 10.—The caudal fin of a swordfish caught in the Gulf of Mexico, showing a crescentic wound and a completed wound cut through the trailing edge of the fin.

found squid remains in most of the stomachs of preserved specimens he inspected, and calculated that the squids which were eaten were as large or larger than the sharks. He wondered how small sharks that apparently swim slowly could catch and capture such large, swift prey.

This question is also pertinent in considering how *Isistius* succeeds in contacting fast-swimming animals such as tunas, marlins, or porpoises. It would appear to be no problem for *Isistius* to approach and make contact with basking or drifting whales or fishes. In the case of tunas, however, there is no evidence that they ever drift or stop swimming (Magnuson, 1970). A possible sequence is that the potential prey, seeing *Isistius* as an object apparently suitable for food, makes the initial approach, identifies it at a short distance, rejects it as food, and veers off. At that instant, the shark may be able to achieve contact by means of a short dash.

It is also possible that the shark, to some degree, simulates other organisms such as squids in the pattern of its luminous ventral surface. A more remote possibility is that *Isistius* is mistaken by large teleosts for a cleaner, and is invited to make contact.

Large squids appear to be killed by *Isistius* more often than merely deprived of plugs of flesh. It may be that squids also make an initial approach but, unlike teleosts, do not veer off from their attack and are subsequently bested in the encounter.

Isouchi (1970) provided the only record of an *Isistius* eaten by a large teleost when he found a living shark in the stomach of *Scomberomorus* sp. This record indicates that *Isistius* is a potential food item; on the other hand, records of teleosts having ingested any species of small or young sharks are limited to five or six (S. Springer and M. R. Bartlett, personal communications). This certainly supports a hypothesis of usual rejection. Rejection of the young as food by teleosts, in fact, may account for the survival of most elasmobranch species, considering their extremely low reproduction rates and relatively low swimming speeds.

It may not be necessary to assume any complicated behavior patterns of *Isistius* or its prey; perhaps contacts by means of short dashes can be made during chance proximities. Thomas Dohl, The Oceanic Institute, Hawaii, has informed me that young porpoises of sizes that are assumed to be still nursing do not bear wounds or scars, but those which are larger do. Similar restriction of wounds and scars to older porpoises is suggested by the data of van Utrecht (1959). This may be simply a matter of an increased probability of encounter with time; but it may, on the other hand, indicate that porpoises are not attacked by *Isistius* until the porpoises become predatory on fish.

Several crescentic wounds which I have examined on tunas were made from a frontal attack

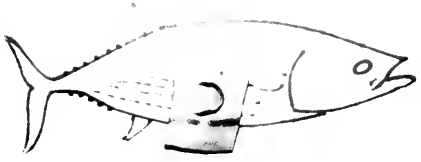

FIGURE 11.—An excised crescentic wound superimposed over a diagram of a skipjack tuna to indicate that the wound was made from a frontal attack, central Pacific.

position, that is, the shark and its prey were going in opposite directions when the wound was inflicted (Figure 11). Such crescentic wounds, as previously pointed out, are apparently the result of circumstances which do not allow the shark to complete the scooping out process. Besides providing support for the suggestion that the teleost makes the initial approach, the evidence of frontal attacks may explain the occurrence of wounds in which the plug of flesh is still attached to the bottom of the wound by a peduncle. Such wounds are common on cetaceans (Mackintosh and Wheeler, 1929; van Utrecht, 1959). In a frontal attack, the drag of water on the shark's body would cause it to rotate, in the manner of the hand of a clock, around the point of attachment until the shark was oriented in the same direction as its prey. This movement would cause the mandibular teeth to act in the manner of a melon-ball cutter and, if penetration was adequate, the crater wound would be completed.

To explore this possibility, I employed a nectarine (*Persicum* sp.) from the ship's galley since no large, dead fish was available at the moment. I pushed the teeth of a fresh, dead *Isistius* into the fruit and then rotated the body around that point. The result (Figure 4) was a neat, round, crater "wound" and the hemispheroidal "plug" in the shark's mouth with the small, hooked upper teeth securing it. If tooth penetration had been inadequate during such a sequence, the integument would be cut completely around but the plug would remain attached by a central peduncle. Necrosis of the plug would probably follow, resulting in conditions described by Mackintosh and Wheeler (1929). They presented a hypothetical sequence beginning with a crescentic wound which developed, by gradual erosion of the flesh, to the open pit stage. The "flabby" pedunculate plug, they believed, was a stage in the healing process and was sloughed off near the completion of healing.

They pointed out that the most obvious cause of crescentic and open pit wounds was the bite of some fish, but no fish known to them possessed teeth or a mouth structure which would produce such wounds. They, therefore, returned to the assumption that the wounds were a result of microbial infections.

Except in the cases of attacks on squids when the prey is killed, it appears that *Isistius*, in biting pieces out of living cetaceans and fishes, qualifies as a temporary parasite in the same sense that a mosquito does.

ACKNOWLEDGMENTS

I want to acknowledge the contributions of many persons who provided unpublished records, specimens of wounds and scars, photographs, as well as ideas and encouragement during the preparation of this manuscript. My special thanks are extended to John D. Fowler, Jr., NMFS, HAFRC, who initiated the important experiments at sea.

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THE RELATIVE IMPORTANCE OF NANNOPLANKTON AND NETPLANKTON AS PRIMARY PRODUCERS IN THE CALIFORNIA CURRENT SYSTEM

THOMAS C. MALONE[1]

ABSTRACT

Nannoplankton and netplankton primary productivity and standing crop were measured on a seasonal basis in Monterey Bay (October 1969 to February 1971) and along four transects of the California Current between lat 35° and 50° N. Nannoplankters accounted for 60 to 99% (mean = 86%) of the observed productivity and standing crop both inshore and offshore under oceanic conditions. Seasonal and geographical variations in the nannoplankton fraction were remarkably stable, and variations in phytoplankton productivity and standing crop were due primarily to the netplankton. The assimilations ratios of both fractions were relatively constant.

Increases in the netplankton fraction were closely coupled with the occurrence of coastal upwelling, and netplankton productivity and standing crop exceeded that of the nannoplankton only during the strongest upwelling pulses. These increases were probably due to the suspension effect of positive vertical advection and to increases in ambient NO_3-N concentrations above 1 to 3 μM. Decreases were in response to increases in grazing pressure and downward water movements. A model is suggested to account for the following observations: (1) the nannoplankton fraction varied within narrow limits compared with the netplankton; (2) nannoplankton assimilation ratios (and presumably growth rates) were consistently high and twice those of the netplankton; and (3) netplankton productivity and standing crop increased relative to the nannoplankton during periods of upwelling. The model is based on the response of particles of varying sinking rates to vertical and horizontal advection, and on the degree of coupling between the production of organic matter by primary producers and grazing by primary consumers.

The phytoplankton can be divided into two size classes based on their retention by fine mesh nets (aperture size 20 to 90 μ). Those retained are commonly called "netplankton" while those which escape are referred to as "nannoplankton." Seasonal and geographic variations in netplankton and nannoplankton primary productivity and standing crop are neither well documented nor understood. Previous investigations in both temperate (Yentsch and Ryther, 1959; McAllister et al., 1959; Gilmartin, 1964; Anderson, 1965) and tropical marine environments (Steeman Nielsen and Jensen, 1957; Holmes, 1958a; Teixeira, 1963; Saijo and Takesue, 1965; Malone, in press a) have demonstrated that the nannoplankton are usually responsible for 80 to 100% of the observed phytoplankton productivity and standing crop. Netplankton productivity is often higher in neritic than in oceanic waters (e.g., Steeman Nielsen and Jensen, 1957; Malone, in press a) but rarely exceeds that of the nannoplankton. However, neritic phytoplankton communities dominated by the netplankton in terms of cell number (Digby, 1953) and chlorophyll concentration (Subrahmanyan and Sarma, 1965) have been reported.

The ecological significance of these two size classes lies in the role of cell size and surface area-to-volume (A/V) ratios in the dynamics of phytoplankton productivity and energy flow through pelagic food chains. Small cells generally have shorter generation times and higher growth rates in a given environment than do larger cells (Findenegg, 1965; Williams, 1965; Eppley and Sloan, 1966; Eppley and Thomas, 1969; Eppley et al., 1969). Recent observations on the kinetics of nutrient uptake by phytoplankton (Eppley et al., 1969) indicate that the half-saturation constants (K_s) for nitrate and ammo-

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nium uptake vary in proportion to cell size, presumably a consequence of the high A/V ratios of smaller cells (Munk and Riley, 1952). Some evidence is also available that maximum uptake rates (V_m), while not species specific, do increase with increasing cell size (Dugdale, 1967; Eppley et al., 1969) so that netplankters with high K_s and V_m values would be favored when nitrate concentrations are high while nannoplankters with low K_s and V_m values would be favored when nitrate concentrations are low.

High A/V ratios facilitate suspension (Munk and Riley, 1952; Smayda and Boleyn, 1966a, b; Eppley et al., 1967) increasing the potential residence times of cells in the photic zone under stratified conditions. Also, since sinking rates generally increase as cell size increases, larger cells will tend to be concentrated in regions of upward water flow while smaller cells will be distributed along a gradient toward regions of downward water flow (Stommel, 1949; Semina, 1968). In this way, small cells will tend to be spread over a greater volume than larger cells, and motile cells seeking to maintain their position in the water column will be concentrated in regions of downward flow (Hutchinson, 1967).

In addition, the distribution of productivity and biomass among different size classes of phytoplankton should be reflected in the distributions and abundances of herbivores which selectively graze on the basis of particle size. Nannoplankters appear to be the preferred food of many planktotrophic larvae (Bruce et al., 1940; Thorson, 1950) and microzooplankton (Beers and Stewart, 1969; Parsons and Le Brasseur, 1970), while herbivorous copepods actively select netplankton species (Harvey, 1937; Mullin, 1963; Conover, 1966; Mullin and Brooks, 1967; Richman and Rogers, 1969). Phytoplankton cell size may also affect the efficiency of energy transfer to large predators, since nannoplankton-based food chains appear to require one or two additional energy transfers to reach a given sized consumer than do netplankton-based food chains (Ryther, 1969; Parsons and Le Brasseur, 1970).

The California Current system and Monterey Bay provide ideal environments in which to study variations in netplankton and nannoplankton productivity and standing crop, since nutrient concentrations and vertical water movements vary markedly both seasonally and geographically. The California Current system is discussed by Reid et al. (1958), and the monthly mean charts of geostrophic flow have been prepared by Wyllie (1966). The southerly flow of the California Current is typically strongest during the spring and summer when northerly winds are best developed. At this time the coastal boundary of the Current is marked by upwelling. During the fall and winter northerly winds are weak or reversed, and a coastal countercurrent (the Davidson Current) often develops between the California Current proper and the coast. Thus, the hydrography of the coastal region off California is generally characterized by upward water movements and high nutrient concentrations during the spring and summer, and downward water movements and low nutrient concentrations during the fall and winter.

The annual cycle of hydrographic conditions in Monterey Bay has been described by Bolin and Abbott (1961) and Bolin (1964). Skogsberg (1936) divided the annual cycle in the upper 100 m into three hydrographic periods:

1. An Upwelling Period (March to September) characterized by low surface temperatures (9.5° to 11.5° C), high salinities (33.2 to 33.9‰), and high nutrient concentrations (>2.0 μM PO_4-P, >5.0 μM NO_3-N, and >10.0 μM SiO_3-Si).
2. An Oceanic Period (September to November) characterized by high surface temperatures (12.0° to 15.0° C), decreasing salinities (33.0 to 33.6‰) and low nutrient concentrations (0.2 to 2.0 μM PO_4-P, 0.0 to 0.5 μM NO_3-N, and 1.0 to 10.0 μM SiO_3-Si).
3. The Davidson Current Period (November to March) characterized by decreasing temperatures (11.0° to 13.5° C), low salinities (32.4 to 33.2‰), and low nutrient conditions.

Water of oceanic origin is brought into the Bay during both Oceanic and Davidson Current Periods, at first passively as the high density upwelled water begins to subside and then actively

when southerly winds prevail. Since both periods are characterized by a downward flux of water (subsidence and downwelling) and low nutrient concentrations in the upper half of the photic zone, they will be consolidated and referred to as the "Oceanic Period."

The purpose of this study is to document temporal and spatial variations in nannoplankton and netplankton productivity and standing crop and to evaluate these variations with respect to dissolved inorganic nitrogen concentrations, vertical water movements, and grazing pressure.

METHODS AND MATERIALS

Measurements of netplankton and nannoplankton primary productivity and standing crop were made at 17 stations in the California Current system between lat 35° and 50° N during July, August, and November 1970, and at California Cooperative Oceanic Fisheries Investigations (CalCOFI) station 3 in Monterey Bay from October 1969 to February 1971 (Figure 1). The latter station is located over the Monterey Submarine Canyon in about 1000 m of water (lat 36°46.8′ N, long 122°01′ W). All data were collected during cruises of the RV *Proteus* (Stanford University).

Netplankton and nannoplankton photosynthetic capacities (rate of carbon fixation as measured by the carbon-14 technique at light saturation), chlorophyll-a concentrations, and cell numbers were estimated from duplicate water samples collected from 2 m below the surface with two Van Dorn bottles. The duplicate Van Dorn bottle samples were taken 3 hr before local apparent noon and again 3 hr after local noon. Four light and two dark bottles (a total of 12 125-ml Pyrex bottles) were drawn from each sample, inoculated with 5 μc of $Na_2{}^{14}CO_3$, and incubated under fluorescent light (about 0.06 langley/min) for 2 to 3 hr at sea-surface temperatures (Doty and Oguri, 1958). Following incubation, half of the light and dark bottles from each Van Dorn sample were fractionated by passing the water first through Nytex-net discs with 22-μ apertures (netplankton) and then through HA Millipore[2] filters (nannoplankton). The remaining four light and two dark bottles were HA Millipore filtered directly as controls. The filter discs were washed with about 30 ml of filtered seawater, dried in a CO_2 free atmosphere, and their activity measured with a Nuclear Chicago scalar (model 161A) equipped with a model D47 gas flow chamber with a micromil window. Each filter was counted for at least 5 min, and rates of carbon fixation were calculated as described by Doty and Oguri (1958) after averaging duplicate light bottle values. Mean coefficients of variation between duplicate light bottles were 6 ± 1% for the nannoplankton and 26 ± 5% (95% confidence limits) for the netplankton. The mean coefficient of variation between phytoplankton productivity values calculated from the sum of the nannoplankton and netplankton fractions and the unfractioned controls was 10 ± 2%.

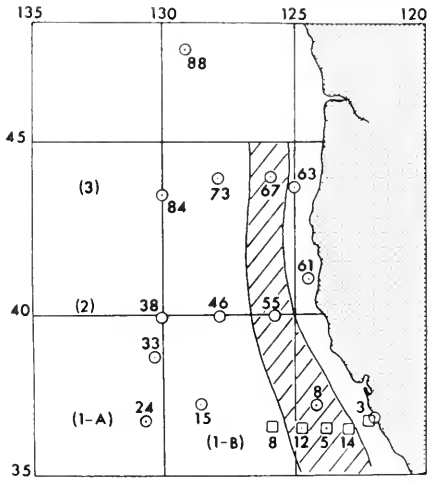


FIGURE 1.—Stations occupied along transects (1-A, B, 2, and 3) of the California Current during July and August (⊙) and during November (□), 1970; the shaded area represents the transition zone between offshore and inshore regions.

[2] The use of trade names is merely to facilitate descriptions; no endorsement is implied.

Samples for pigment analysis were also collected 2 to 3 hr before local apparent noon from 13 depths between the surface and 100 to 200 m. The upper 6 to 10 depths sampled were within the photic zone, depending on its depth, and were chosen on the basis of the thermal structure of the water column. Sample depths were evenly spaced through the mixed layer and evenly but more closely spaced across the thermocline. Samples were always taken at the base of the photic zone and at two depths below to at least twice the photic zone depth. Chlorophyll-a and phaeopigment concentrations were determined by a fluorometric technique (Strickland and Parsons, 1968). Water samples were fractionated by the same procedure described for the carbon-uptake experiments except Whatman GF C glass fibre filters coated with 2 ml of 1% $MgCO_3$ suspension were used in place of membrane filters, and the netplankton chlorophyll fraction was calculated from the difference between fractionated and unfractionated values. Duplicate values for each fraction were averaged (mean coefficients of variation were 10 ± 2% for the nannoplankton and 22 ± 8% for the netplankton fraction). The use of glass filters may have led to no more than a 10% underestimation of nannoplankton chlorophyll-a (Malone, in press a).

Samples for phytoplankton enumeration and identification were preserved with Lugol's solution made basic with sodium acetate in place of acetic acid. Aliquots of 100 ml were placed in Nessler tubes and the cells allowed to settle for 72 hr. Depending on the concentration of cells, from 50 to 90 ml of the supernatant was then siphoned off and 2 ml aliquots were added to settling chambers. After 18 hr the samples were counted by the inverted microscope technique of Utermöhl (Lund et al., 1958). All organisms longer than about 30 μ were counted at 100 × while smaller cells were counted in 100 random fields at a magnification of 100 ×. For lack of better criteria, phytoplankton having dimensions of 30 × 22 μ or less were classified as nannoplankton and those with larger dimensions as netplankton. This did not present much of a problem, however, because the nannoplankton fraction was dominated by cells whose longest dimension was in the range of 2 to 15 μ, while the netplankton fraction was dominated by chain-forming diatoms with cell lengths of 40 μ or more, e.g., *Nitzschia pacifica*. Dominant netplankton forms were identified to species, and less numerous forms to genus. The remaining phytoplankters were classified as pennate or centric diatoms, thecate or nonthecate dinoflagellates, coccolithophores, silicoflagellates, or "others." Mean coefficients of variation between duplicate samples were 14 ± 4% for the nannoplankton fraction and 27 ± 11% for the netplankton fraction.

Standard hydrographic and bathythermograph casts were made 2 to 4 hr before local apparent noon in conjunction with productivity and standing crop measurements to estimate the vertical distributions of dissolved inorganic nitrogen compounds, temperature, and density in the water column. Additional hydrographic casts made for the CalCOFI Program in Monterey Bay are utilized in this paper. Nitrate concentrations were determined by the manual procedure described by Strickland and Parsons (1968) and ammonium by the phenolhypochlorite method (Solorzano, 1969). A Secchi disc was used to estimate photic zone depths (3.5 × Secchi disc reading).

The ratio of phaeopigments-to-chlorophyll in the water column (to 100 m for inshore stations and to 200 m for offshore stations) was used as a rough index of relative grazing pressure on the phytoplankton standing crop (Lorenzen, 1967; Beers and Stewart, 1969). In the present study, a highly significant ($P = 0.01$) regression of phaeopigment concentration on $\log_{10}$ transformed zooplankton wet weights was found, and it was concluded that the phaeopigment-chlorophyll ratio could be used as a first order index of grazing pressure.

TEMPORAL VARIATIONS IN MONTEREY BAY

ENVIRONMENTAL FACTORS

The hydrographic conditions observed at CalCOFI 3 from October 1969 to February 1971 are summarized in Figure 2 and Table 1. Sur-

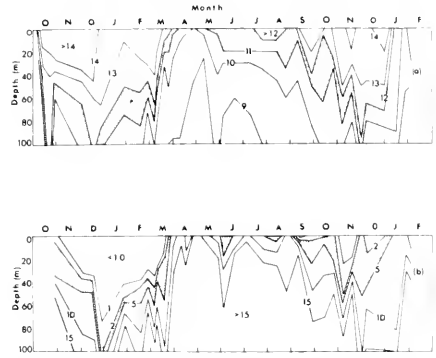


FIGURE 2.—a. Vertical distribution of temperature (°C) at CalCOFI 3 from October 1969 to February 1971. b. Vertical distribution of NO_3-N (μM).

TABLE 1.—Environmental factors monitored at the surface, concurrently with measurements of productivity and standing crop at CalCOFI station 3 in Monterey Bay.

Date	Temperature	Salinity	NO_3-N	NH_4-N	Mixed layer	Photic zone
	°C	‰	μM	μM	m	m
28 Oct. 69	14.20	33.51	1.7	--	30	60
2 Dec.	14.68	33.39	0.3	--	30	55
26 Feb. 70	13.10	32.68	0.3	0.3	50	60
5 Mar.	13.20	32.99	0.4	0.6	30	50
10 Mar.	13.17	32.98	0.1	0.2	25	65
18 Mar.	12.06	33.11	0.4	0.4	15	30
31 Mar.	11.17	33.49	7.8	0.3	0	25
8 Apr.	10.45	33.65	14.5	0.1	30	45
18 June	12.44	33.81	6.5	0.5	10	15
2 July	11.02	33.71	13.9	1.5	0	40
26 July	12.95	33.82	6.5	3.3	0	40
12 Oct.	13.56	33.53	1.1	0.6	0	70
26 Oct.	13.03	33.41	3.1	0.8	30	65
6 Nov.	13.59	32.99	0.7	0.2	40	65
18 Nov.	14.29	33.26	0.2	--	15	60
30 Nov.	13.40	33.28	2.4	--	45	55
7 Dec.	13.24	33.02	1.3	--	50	55
17 Jan. 71	11.50	33.43	6.6	0.4	50	60
27 Jan.	10.84	33.52	8.7	0.8	20	30
3 Feb.	11.79	33.33	3.8	0.4	15	50

face water of oceanic origin was found in the Bay from October 1969 to mid-March 1970. The intrusion of oceanic water and the general subsidence of the water mass are evidenced by the descending isotherms and nitrate isopleths, high surface temperatures, and low salinities. Mixed layer and photic zone depths were about 30 and 60 m respectively, and NO_3-N concentrations were less than 0.5 μM throughout most of the photic zone.

Upwelling was initiated in March as indicated by the ascending isotherms and nitrate isopleths, NO_3-N concentrations in excess of 5.0 μM over the entire photic zone, low surface temperatures, and high surface salinities. With the exception of a minor intrusion of oceanic water late in July and early August, upwelling continued uninterrupted into September with peaks in early April and early September. "Mixed layer" depths varied between 0 and 30 m, and at no time exceeded the depth of the photic zone, which ranged from 15 to 45 m.

From September through December the hydrography of the Bay was confused and neither oceanic nor upwelling conditions ever predominated. Weak upwelling surges bracketed by influxes of oceanic water occurred during late October and late November (Figure 2). Surface NO_3-N concentrations were variable (0.2 to 3.1 μM) reflecting the indecisiveness of the system. Then, following a period of oceanic water during December and early January, a strong upwelling pulse occurred which was about as intense as the upwelling during late March and early April of the previous year.

Hydrographically, three periods can be distinguished in the Bay during the period of this study:

1. A stable Oceanic Period from October 1969, into March 1970,
2. A stable Upwelling Period from March into September,
3. A "Mixed" Period from September through December punctuated by a strong upwelling pulse in January.

The grazing pressure index declined during the transition from oceanic to upwelling conditions to a low of 0.03 in early April (Figure 3). Grazing pressure then increased rapidly during the steady upwelling of June and July to an annual maximum of 2.03 which was followed by a gradual decline during the Mixed Period ending with a sharp rise in late January to a peak in early February.

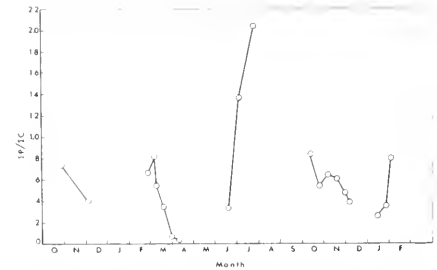


FIGURE 3.—Temporal variations in the ratio of phaeopigments-to-chlorophyll-a (P/C) integrated over the upper 100 m at CalCOFI 3.

SURFACE PRIMARY PRODUCTIVITY AND STANDING CROP

Seasonal variations in surface productivity and standing crop are shown in Figure 4. Since a significant difference (F-test, $P<0.01$) was not observed between morning and afternoon values (see Malone, in press b), only data collected during the morning sampling will be considered.

Phytoplankton productivity and standing crop remained below 5.0 mgC m^{-3} hr^{-1}, 1.00 mgChl-a m^{-3}, and 3.5 $\times$ 10^5 cells/liter during the Oceanic Period. Values above 10.0, 1.50, and 10.0 $\times$ 10^5 were observed only during the Upwelling Period and the January Upwelling pulse. The Mixed Period was characterized by intermediate levels of productivity and standing crop. Three peaks were observed, of which the two greatest coincided with the two most intense upwelling pulses: (1) 50.4 mgC m^{-3} hr^{-1}, 7.92 mgChl-a m^{-3}, and 24.2 $\times$ 10^5 cells/liter on the last day of March, and (2) 43.4, 10.46, and 23.9 $\times$ 10^5 during the last week of January. The third, less pronounced peak, was in mid-June during steady upwelling.

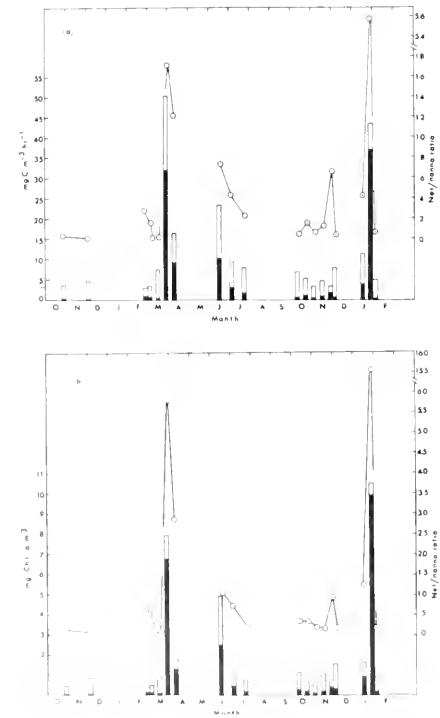
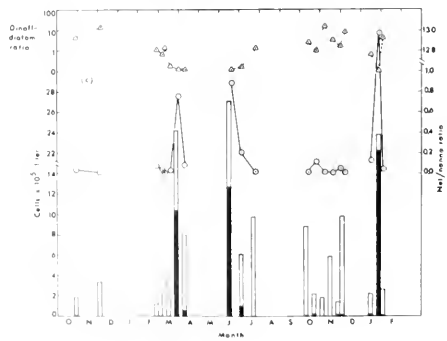


FIGURE 4.—a. Temporal variations in surface netplankton (■) and nannoplankton (□) productivity (mgC m^{-3} hr^{-1}) and the net/nanno ratio (○) at CalCOFI 3. b. Temporal variations in surface chlorophyll-a (mg m^{-3}) and the net/nanno ratio. c. Temporal variations in surface cell numbers, the net/nanno ratio, and the ratio of dinoflagellates-to-diatoms (△).



Phytoplankton assimilation ratios (mgC hr^{-1} mgChl-a^{-1}) were relatively constant, with most values falling between 5 and 10 (mean = 7.4 ± 1.0, 95% confidence limits). Fluctuations in the amount of chlorophyll-a per cell (10^{-6} μg) were also within comparatively narrow limits. Values varied from 0.85 to 6.97 with a mean of 2.62 ± 0.66.

Surface levels of nannoplankton productivity and standing crop were remarkably stable through the year. Productivity and standing crop values were less than 8.0 mgC m^{-3} hr^{-1}, 0.80 mgChl-a m^{-3}, and 3.3 × 10^5 cells/liter during the Oceanic Period. During the Upwelling Period productivity ranged from 6.6 to 18.6, chlorophyll-a from 0.46 to 2.44, and cell numbers from 5.1 to 14.4 × 10^5. Thus, while nannoplankton productivity and standing crop were lower under oceanic than upwelling conditions, the differences were not marked.

In contrast, netplankton productivity and standing crop varied tremendously during the year, from less than 0.6 mgC m^{-3} hr^{-1}, 0.14 mgChl-a m^{-3}, and 0.1 × 10^5 cells/liter during the Oceanic Period to greater than 2.8, 0.40, and 0.6 × 10^5 during the Upwelling Period. Two prominent peaks were observed (31.8 mgC m^{-3} hr^{-1}, 6.76 mgChl-a m^{-3} and 36.8 and 9.83), both in association with the two most intense upwelling pulses. A secondary peak occurred in mid-June. Netplankton cell numbers reached successive peaks of 24.2, 27.1, and 23.8 × 10^5 cells/liter which coincided with peaks in productivity and chlorophyll-a. During the fall and early winter Mixed Period, intermediate values were observed with small peaks associated with each short burst of upwelling. Thus, netplankton productivity and standing crop varied from an order of magnitude less than that of the nannoplankton during the Oceanic Period to an order of magnitude greater during the Upwelling Period. Comparison of mean squares and ranges of variation (Table 2) clearly demonstrates that temporal variations in phytoplankton productivity and standing crop were primarily due to the netplankton fraction with the nannoplankton maintaining a relatively stable background level.

Variations in the ratio of netplankton-to-nannoplankton (net/nanno) are also shown in Figure 4. The net/nanno productivity ratio never exceeded 0.3 during intrusions of oceanic water (either during the Oceanic Period or the Mixed Period), and was greater than 1.0 on only two occasions: during the strong upwelling pulses of late March and late January. The same pattern was found for the net/nanno chlorophyll and cell number ratios except the chlorophyll ratios were consistently higher and the cell number ratios lower than the productivity ratios. This is reflected in the assimilation ratios and cell chlorophyll-a content of the two fractions, both of which were relatively constant during the study. The mean nannoplankton assimilation ratio of 9.4 ± 1.5 was significantly higher than the netplankton mean of 4.7 ± 1.3. Similarly, the nannoplankton had more cells per unit chlorophyll-a than did the netplankton. The mean chlorophyll-a content per netplankton cell was 23.6 ± 13.1 × 10^{-6} μg which is significantly higher than the nannoplankton mean of 1.9 ± 0.5 × 10^{-6} μg.

Peaks in the ratio of netplankton-to-nannoplankton cell numbers coincided with peaks in netplankton cell number, but the ratio exceeded 1.0 only during the January bloom. This probably reflects the dominance of the small-celled (<20 μ in length), chain-forming diatoms *Chaetoceros socialis* and *Skeletonema costatum* in the netplankton fraction. In contrast, the netplankton blooms of late March and mid-June were dominated by large-celled (>40 μ in length) chain-forming diatoms *Nitzschia pacifica* and *Rhizosolenia fragilissima*, respectively. *Nitzschia* spp., *Skeletonema costatum*, *Leptocylindricus* sp., and *Chaetoceros* spp. accounted for

TABLE 2.—Mean squares and range factors (maximum/minimum) for nannoplankton and netplankton productivity (PP = mgC m^{-3} hr^{-1}), chlorophyll-a concentration (mg m^{-3}, m^{-2}), and cell numbers (no./liter) at CalCOFI station 3.

Fraction	Mean squares			
	PP	*mg m⁻³*	*no./liter*	*mg m⁻²*
nannoplankton	16	0.2	17	86
netplankton	109	6.5	32	1,680
	Range factors			
nannoplankton	9	12	13	4
netplankton	1,800	4,200	4,400	120

70% of the netplankton in the March bloom. In mid-June *Rhizosolenia* spp. and *Nitzschia* spp. made up 80% of the netplankton. The nannoplankton fraction was dominated by small monads 2 to 15 μ in length in all but one of the samples examined. The one exception occurred at the peak of the March-April netplankton bloom when small diatoms dominated the nannoplankton fraction. When the net/nanno ratio was high (during upwelling), diatoms were more numerous than dinoflagellates; but when the ratio was low (during oceanic conditions), dinoflagellates were more numerous (Figure 4c).

VERTICAL DISTRIBUTION OF PIGMENTS

The chlorophyll-a content of the water column (0 to 100 m) varied between 14 and 30 mg m^{-2} during the Oceanic Period and between 24 and 152 mg m^{-2} during the Upwelling Period (Figure 5). The seasonal pattern of variation was much the same as that observed for surface chlorophyll concentrations, but the range of variations was less.

Variations in netplankton and nannoplankton chlorophyll content of the water column were also similar to the surface pattern. Nannoplankton chlorophyll-a values fluctuated between the low of 9.6 mg m^{-2} observed during the Oceanic Period and the high of 44.2 observed during the Mixed Period. Water column levels of netplankton chlorophyll-a, however, were less than 8.0 during the Oceanic Period and surpassed 110 during both strong upwelling pulses. Again, changes in the phytoplankton chlorophyll content of the water column and in the net/nanno ratio were due primarily to variations in the netplankton fraction with the nannoplankton fraction remaining comparatively constant (Table 2).

The vertical distribution of chlorophyll-a always exhibited a maximum which was in the photic zone above or in association with the phaeopigment maximum. The netplankton maximum was always located below the nannoplankton maximum except during strong upwelling when both maxima occurred in the upper 10 m of the photic zone (Figure 6). Four stations have been selected to illustrate the different types of vertical pigment distributions encountered (Figure 7). Two basic patterns were observed, a stable oceanic distribution with low chlorophyll concentrations and low net/nanno ratios (Figure 7a), and an upwelling distribution with high chlorophyll concentrations and high net/nanno ratios (Figure 7b). Under oceanic conditions, the nannoplankton maximum was found in the upper half of the photic zone, near the bottom of the mixed layer and in nitrate-poor water

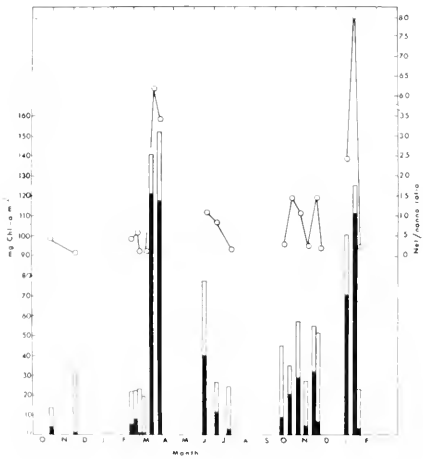


FIGURE 5.—Temporal variations in netplankton (■) and nannoplankton (—) chlorophyll-a content of the water column (mg m^{-2}, 0 to 100 m) and the net/nanno ratio ( ) at CalCOFI 3.

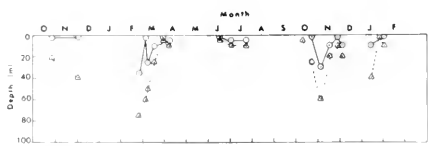


FIGURE 6.—Temporal variations in the depths of the nannoplankton ( ) and netplankton (△) chlorophyll-a maxima at CalCOFI 3.

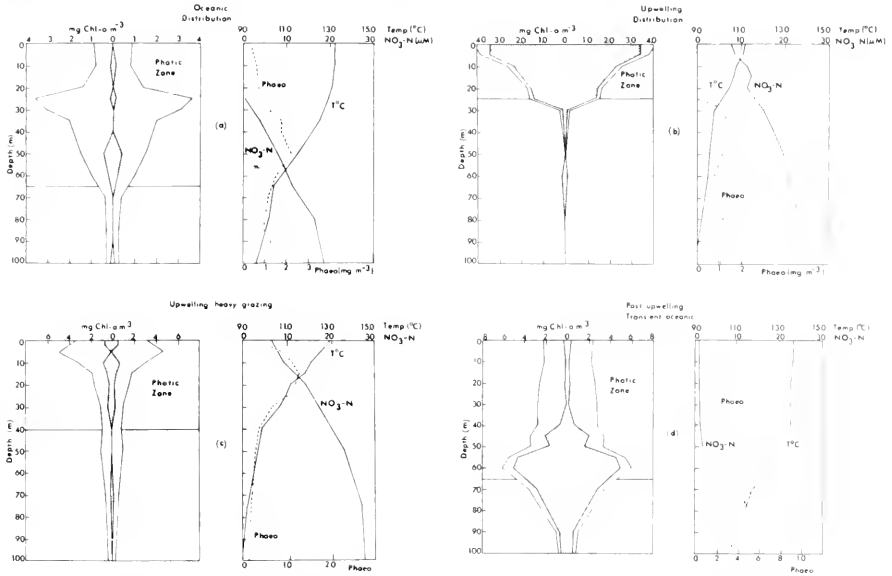


FIGURE 7.—Vertical profiles of netplankton (□) and nannoplankton (□) chlorophyll-a (mg m^{-3}), NO_3-N (μM), and temperature (°C) at CalCOFI 3: (a) Oceanic Period (10 March), (b) Upwelling Period (31 March), (c) Upwelling Period with peak grazing pressure (26 July), and (d) Mixed Period during post-upwelling subsidence (6 November).

(<0.5 μM NO_3-N); while the netplankton maximum was located in the lower half of the photic zone, in the thermocline, and in nitrate-rich water (>5.0 μM NO_3-N). Maximum phaeopigment concentrations occurred in association with or just below the netplankton maximum. With the onset of upwelling, the netplankton maximum gradually shifted from a depth of 75 m to the surface (Figure 6). Initially, upwelling had a dilution effect which was followed by a rapid increase in the netplankton fraction and later by a slight increase in the nannoplankton fraction (Figure 5). The upwelling distribution observed on March 31 is shown in Figure 7b. Both netplankton and nannoplankton chlorophyll maxima were at the surface and nitrate concentrations were high (>5.0 μM NO_3-N) throughout the photic zone.

The remaining two examples represent special cases which evolved from an upwelling distribution such as the one just described. Figure 7c shows the distribution observed during late July that developed over the period of steady upwelling during which the grazing pressure index increased markedly (Figure 3). Note that both netplankton and nannoplankton maxima were in the upper 10 m, and NO_3-N concentrations were in excess of 5 μM throughout the photic zone; but that the concentration of netplankton chlorophyll has been greatly reduced and the net/nano ratio was low. Phaeopigments were high with a maximum just below the netplankton chlorophyll maximum. The distribution shown in Figure 7d (November 6) developed during a period of subsidence following an upwelling pulse. At this time, the netplankton maximum

was near the bottom of the photic zone 30 m below the nannoplankton maximum; the netplankton chlorophyll concentration and net/nanno ratio were still high; and NO_3-N concentrations were greater than 2 μM throughout most of the photic zone.

GEOGRAPHIC VARIATIONS IN THE CALIFORNIA CURRENT

ENVIRONMENTAL FACTORS

Four transects across the core of the Current were made for this study (Figure 1). Transects 1-A, 2, and 3 were made during late July and August 1970, and transect 1-B was made during the first week of November 1970. CalCOFI station 3 in Monterey Bay was the inshore station for transects 1-A and B. The hydrographic conditions observed along these transects are summarized in Table 3 and Figures 8 and 9.

The July-August transects were made during a period of coastal upwelling and were characterized by shoreward rising isotherms and nitrate isopleths. Based on the upward slope of these isopleths, upwelling was least intense at the southernmost inshore station and most intense at the northernmost station, which is typical for this time of year (Reid et al., 1958). Nitrate concentrations were high in the upper half of the photic zone at the three inshore stations and low at the two outermost stations of each transect. Ammonium concentrations were relatively high (>1 μM NH_4-N) in the photic zone at the stations of transect 1-A but were low (typically 0.1 to 0.5 μM) throughout the water column at most of the remaining stations. The surface mixed layer was never observed to extend below the photic zone, inshore or offshore. An undercurrent was present below the thermocline between stations 5 and 8 of transect 1-A as indicated by the spreading isotherms (cf. Wooster and Gilmartin, 1961). Based on the temperature (Figure 8) and nitrate profiles (Figure 9), the stations along each transect were divided into three groups:

1. Stations within about 100 km of the coast were classified as inshore (stations 3, 61, and 63),
2. Stations between 100 and 250 km offshore were classified as transitional (stations 8, 55, and 67),

TABLE 3.—Environmental factors monitored concurrently with measurements of productivity and standing crop in the California Current system between lat 35° and 50° N.

Station	Date	Distance from land	Temperature	Salinity	NO_3-N	NH_4-N	Mixed layer	Photic zone
		km	*°C*	*‰*	*μM*	*μM*	*m*	*m*
03a	26 July 70	15	12.95	33.82	6.5	3.3	0	40
08	27 July	155	14.88	33.11	1.3	1.4	20	65
15	29 July	470	17.69	32.95	0.2	1.8	30	105
24	31 July	675	18.49	32.88	0.1	1.7	20	100
33	2 Aug.	535	18.03	32.51	0.0	0.4	0	115
61	9 Aug.	30	11.98	33.38	8.8	0.7	10	40
55	7 Aug.	130	16.04	31.88	0.1	0.0	10	65
46	5 Aug.	310	17.25	32.08	0.1	0.4	15	105
38	3 Aug.	485	18.25	32.79	0.1	0.3	15	95
63	15 Aug.	70	11.55	33.00	0.3	0.5	10	15
67	16 Aug.	150	14.91	32.62	0.7	0.2	30	75
73	18 Aug.	315	17.24	32.04	0.1	0.5	20	55
84	20 Aug.	450	17.07	32.57	0.2	0.1	30	90
88	22 Aug.	280	15.82	32.47	0.1	0.3	20	80
03a	6 Nov.	15	13.59	32.99	0.7	0.2	40	60
14	5 Nov	90	14.58	32.98	0.0	0.5	20	65
05	2 Nov	150	14.38	32.81	0.1	0.7	20	50
12	4 Nov.	225	14.74	32.62	0.2	0.1	15	60
08	3 Nov.	290	15.31	32.46	0.1	1.0	15	60

a CalCOFI station 3.

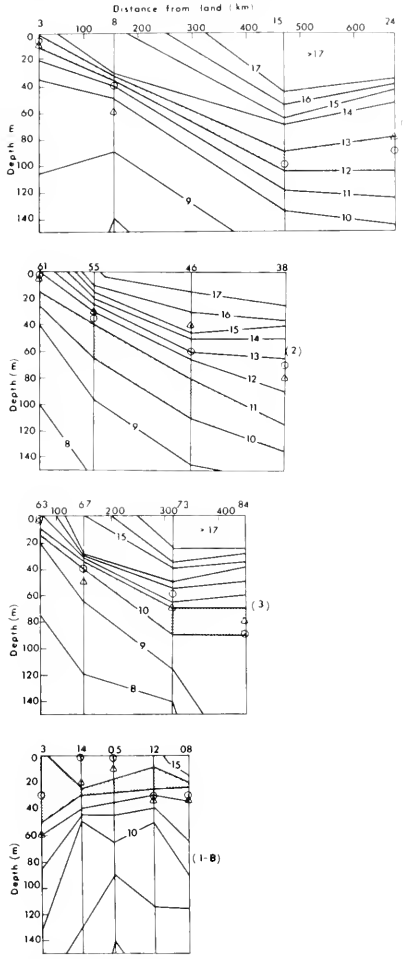


FIGURE 8.—Vertical distribution of temperature (°C) along four transects of the California Current system with the depths of the nannoplankton (○) and netplankton (△) chlorophyll-a maxima: (1-A) along lat 37° N in July, (2) along lat 40° N in August, (3) along lat 44° N in August, and 1-B along lat 36°40' in November. Vertical lines represent stations.

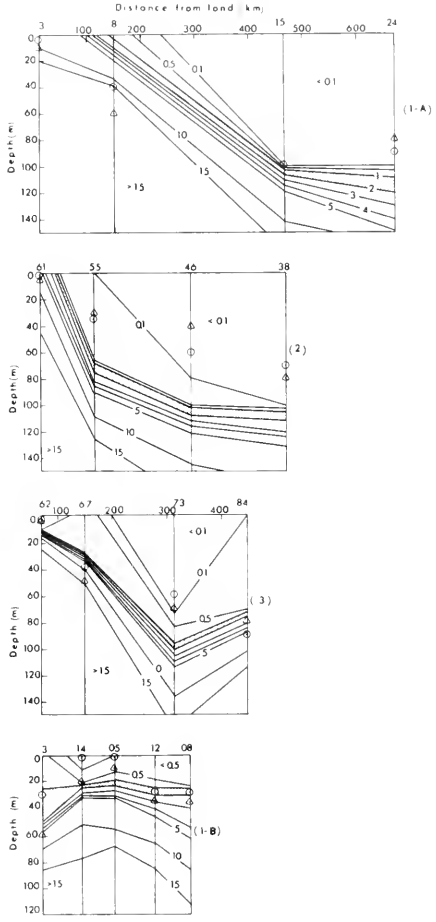


FIGURE 9.—Vertical distribution of NO_3-N (μM) along four transects of the California Current system with the depths of the nannoplankton (○) and netplankton (△) chlorophyll-a maxima.

3. Stations greater than 250 km from the coast were classified as offshore (stations 15, 24, 33, 46, 38, 73, 84, and 88).

Transect 1-B was made at a time when the Davidson Current is usually developed (Bolin, 1964). The inshore station (CalCOFI 3) was occupied during an oceanic phase of the Mixed Period, and subsidence is evidenced by the downward trend of the isotherms and nitrate isopleths (Figures 2, 8, and 9). Surface temperatures were comparatively high and nitrate concentrations low. The Davidson Current was developed at stations 3 and 14, and the transition from Davidson to California Current Water occurred between stations 14 and 12 with a surface divergence probably located between stations 5 and 12. Station 8, the outermost station, was in the California Current proper.

SURFACE PRIMARY PRODUCTIVITY AND STANDING CROP

During the July-August transects, when coastal upwelling dominated the hydrographic regime of the California Current system, phytoplankton productivity and chlorophyll-a concentrations decreased markedly with distance from land (Figure 10). Inshore, productivity and chlorophyll ranged from 6.62 to 61.65 mgC m^{-3} hr^{-1} and from 0.8 to 11.5 mg m^{-3}, respectively. Pro-

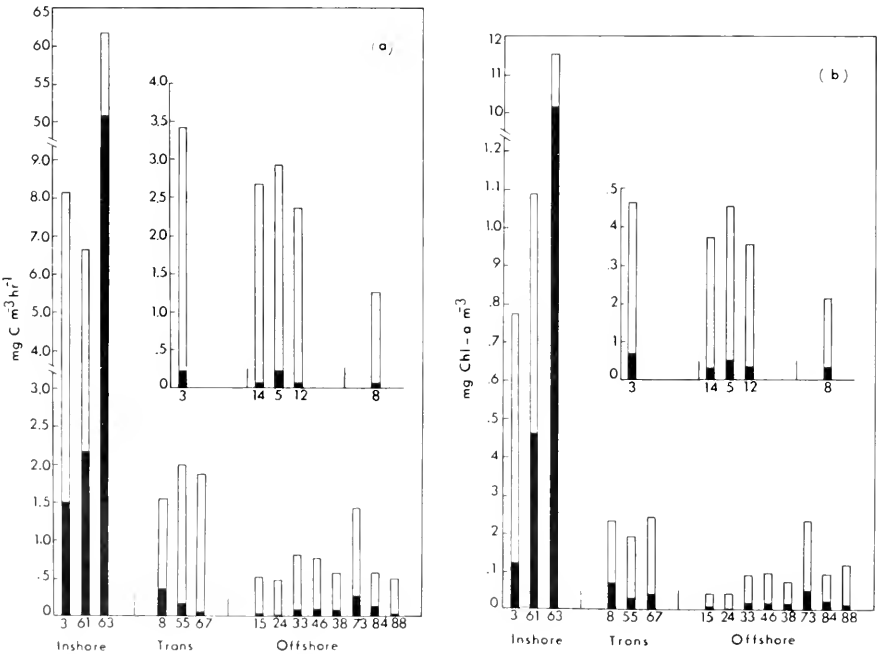


FIGURE 10.—a. Inshore-offshore variations in surface netplankton (■) and nannoplankton (□) productivity (mgC m^{-3} hr^{-1}) during the July-August transects and the November transect (inset). b. Inshore-offshore variations in surface netplankton and nannoplankton chlorophyll-a (mg m^{-3}).

ductivity and chlorophyll concentrations offshore, however, exceeded 1.0 mgC m^{-3} hr^{-1} and 0.10 mgChl-a m^{-3} only once. The highest levels of productivity and chlorophyll were found at inshore station 63 and were equivalent to the maximum values observed at CalCOFI 3 during the two most intense upwelling pulses.

This inshore-offshore decrease in surface productivity and chlorophyll was not observed over the first 225 km of the November transect when subsidence rather than upwelling characterized the coastal hydrographic regime. Productivity and chlorophyll concentrations were relatively constant out to station 12 (Figure 10) and corresponded with the minimum values observed at CalCOFI 3 during the Oceanic Period.

Both nannoplankton and netplankton productivity and chlorophyll decreased markedly between inshore and offshore stations along the July-August transects (Figure 10). Nannoplankton values fell by as much as an order of magnitude from above 4.0 mgC m^{-3} hr^{-1} and 0.60 mgChl-a m^{-3} to less than 1.2 and 0.18, respectively. The netplankton, however, exhibited the greatest decline. Netplankton productivity decreased by 2 to 3 orders of magnitude from 1.5 to 51.3 mgC m^{-3} hr^{-1} to offshore levels of 0.01 to 0.26. Similarly, netplankton chlorophyll values were 0.12 to 10.14 mg m^{-3} inshore and 0.002 to 0.052 offshore. This decline in the netplankton fraction relative to the nannoplankton is reflected in the net nanno ratios (Table 4). Inshore productivity ratios ranged from 0.23 to 4.95, while offshore ratios varied from 0.02 to 0.36. Chlorophyll ratios followed the same pattern but tended to be higher.

The pattern observed in November was quite different. Levels of netplankton productivity and chlorophyll were low along the entire transect and were within the range commonly found offshore and during oceanic phases inshore (Figure 10). Nannoplankton productivity declined slightly from an inshore maximum of 3.20 to an offshore minimum of 1.21. Variations in surface chlorophyll were similar except the maximum of 0.40 was observed at station 5 which is 150 km from shore. Station 5 is particularly interesting because netplankton productivity and chlorophyll also exhibited small peaks here, and it was located near the boundary between the Davidson and California Currents which is marked by a surface divergence and associated upwelling. In this connection, it is also noteworthy that nannoplankton productivity and chlorophyll levels were about twice those observed previously for transitional and offshore regions.

Phytoplankton assimilation ratios were similar to those observed at CalCOFI 3, most values falling between 5 and 10. Excluding inshore stations, the mean assimilation ratio was 7.7 ± 1.1, which is not significantly different from the mean observed at CalCOFI 3. Nannoplankton ratios averaged 8.3 ± 1.2 which is twice the observed mean netplankton ratio of 4.1 ± 0.8. Both means are equivalent to those observed at CalCOFI 3.

VERTICAL DISTRIBUTION OF PIGMENTS

Inshore-offshore variations in the chlorophyll-a content of the water column (0 to 200 m) during the July-August transects were similar in trend but less in amplitude than that observed at the surface. Inshore, chlorophyll varied from 27.32 to 217.68 mg m^{-2} compared with the

TABLE 4.—Netplankton-nannoplankton ratios in the California Current system: primary productivity (PP = mgC m^{-3} hr^{-1}), chlorophyll-a m^{-3}, and chlorophyll-a m^{-2}.

Station	PP	m^{-3}	m^{-2}
03	0.23	0.18	0.14
08	0.30	0.48	0.36
15	0.06	0.15	0.21
24	0.02	0.05	0.14
33	0.11	0.22	0.06
61	0.49	0.75	0.75
55	0.09	0.19	0.16
46	0.12	0.22	0.31
38	0.19	0.27	0.24
63	4.95	7.31	5.82
67	0.03	0.18	0.33
73	0.22	0.28	0.22
84	0.36	0.32	0.14
88	0.07	0.09	0.09
03	0.07	0.17	1.12
14	0.02	0.10	0.18
05	0.08	0.12	0.20
12	0.03	0.11	0.10
08	0.04	0.17	0.16

offshore range of 10.72 to 25.96 mg m^{-2} (Figure 11). In November the pattern was much the same, with an inshore maximum of 66.64 and an offshore level of 23.20.

Nannoplankton chlorophyll in the water column showed little variability (Figure 11). Inshore levels of nannoplankton chlorophyll varied from 17.49 to 31.94 mg m^{-2}, while offshore levels ranged between 9.63 and 21.76. The netplankton fraction underwent much greater fluctuations (Figure 11). Inshore concentrations ranged between 3.26 and 185.74 in contrast to the offshore range of 1.00 to 4.97. The latter range is equivalent to that observed at CalCOFI 3 during the Oceanic Period and the former to that observed during the Upwelling Period. Excluding inshore stations, the mean chlorophyll-a concentration of the nannoplankton fraction was 16.55 ± 2.38 which is significantly higher ($P = 0.05$) than the netplankton mean of 3.08 ± 0.81.

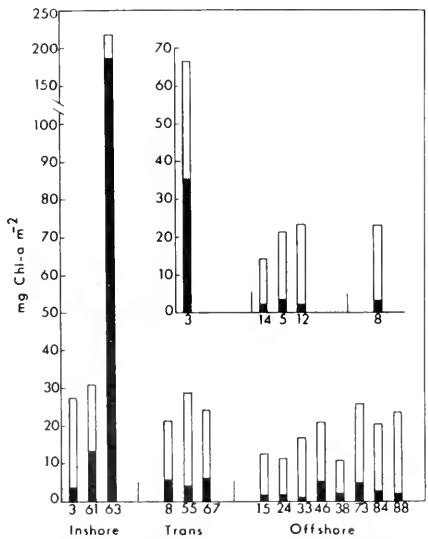


FIGURE 11.—Inshore-offshore variations in the netplankton (■) and nannoplankton (□) chlorophyll-a content of the water column (mg m^{-2}, 0 to 200 m) during the July-August transects and the November transect (inset).

The vertical distributions of chlorophyll-a and phaeopigments at the offshore stations of the July-August transects were characterized by a subsurface maximum located at the base of the photic zone, in the lower part of the thermocline, and near the upper reaches of the nitrate-rich layer (Figure 12). Netplankton and nannoplankton chlorophyll maxima were usually located near each other, but the netplankton maximum was not always deeper than the nannoplankton maximum. Netplankton chlorophyll was more evenly distributed and concentrations were much lower than in inshore waters (compare Figures 7 and 12). Both maxima gradually decreased in depth shoreward from between 80 and 100 m offshore to 10 m or less at the inshore stations paralleling the upward trend of the isotherms (Figure 8) and nitrate isopleths (Figure 9).

The pattern was much different during the November transect (1-B). The netplankton maximum was always located below the nannoplankton maximum, especially at the inshore station where the netplankton maximum was 30 m below the nannoplankton maximum (Figure 7d). Both maxima decreased in depth seaward to station 5 (Figures 8 and 9) where the vertical distribution conformed to the upwelling distribution (Figure 12), i.e., netplankton and nannoplankton maxima were in the upper 10 m and nitrate concentrations were relatively high throughout most of the photic zone. Farther offshore the depth of the chlorophyll maxima increased once again. This up and down movement of the maxima closely paralleled the depth variation of the isotherms and nitrate isopleths just as during the July-August transects.

DISCUSSION

The constancy of the phytoplankton assimilation ratios both inshore (7.4 ± 1.0) and offshore (7.7 ± 1.1) suggests that nutrients were rarely limiting to primary productivity (Dickman, 1969). The mean assimilation ratios found are close to the value of 7.3 reported by Holmes (1958b) in the nutrient-rich waters of the Costa

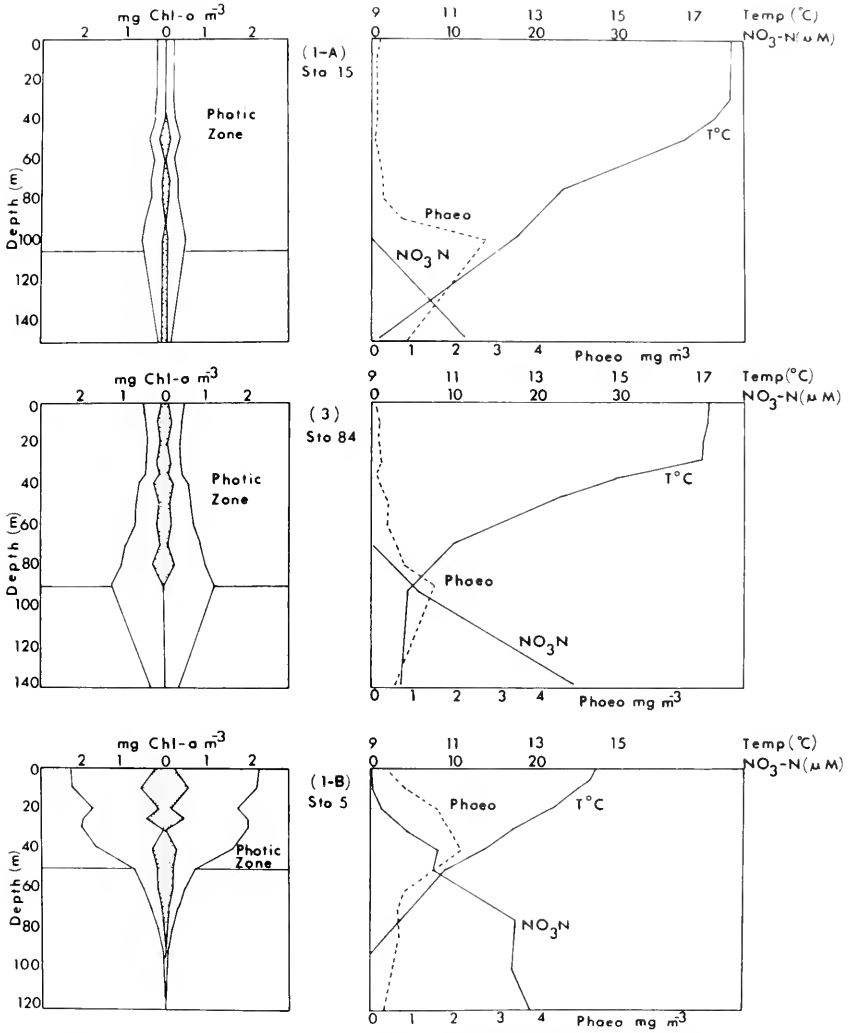


FIGURE 12.—Vertical profiles of netplankton (□) and nannoplankton (■) chlorophyll-a (mg m^{-3}), phaeopigments (mg m^{-3}), NO_3-N (μM), and temperature (°C): transect 1-A, station 15, offshore region; transect 3, station 84, offshore; transect 1-B, station 5, transition zone.

Rica Dome (incubator light intensity about 0.06 langley/min) and are not significantly different from the mean of 8.6 ± 1.3 found by Curl and Small (1965) at light saturation based on in situ measurements. Anderson (1964), working off the Washington and Oregon coasts, obtained ratios of 1.6 to 9.8 (at about 0.02 langley min) with low values occurring during the summer when nutrient concentrations were low and high values during the spring bloom when nutrient concentrations were high. In the eastern tropical Pacific, Thomas (1970) and Malone (in press a) found that assimilation ratios were significantly less in nitrogen-poor than in nitrogen-rich waters. These results are consistent with the observations of Curl and Small (1965), supported by McAllister et al. (1964), which suggest that ratios below 3 are indicative of a nutrient deficiency while those above 5 indicate nutrient-rich waters.

Both the nannoplankton and the netplankton exhibited relatively constant assimilation ratios, but mean nannoplankton ratios were significantly higher (9.4 ± 1.5 inshore, 8.3 ± 1.2 offshore) and twice as great as those of the netplankton (4.7 ± 1.3 inshore, 4.1 ± 0.8 offshore). The constancy of these ratios over a wide range of productivity values in spite of large variations in ambient nitrogen concentrations indicates that nutrient concentration was not an important limiting factor and that the phytoplankton were adapted to about the same light intensity over the entire year. This is conceivable since seasonal variations in day length and light intensity tend to be dampened by the seasonal pattern of cloud coverage, i.e., the summer months are usually foggy while the winter months are usually clear. The situation is similar to that found off La Jolla (Strickland et al., 1970).

Increases in the productivity and standing crop of the netplankton fraction and in the net : nanno ratio were closely coupled with the occurrence of upwelling. Each new upwelling pulse, regardless of duration (CalCOFI 3) or location (transect 1-B) was marked by an increase in net : nanno ratios and netplankton standing crop. Potentially, upwelling can affect phytoplankton productivity in at least two ways: (1) by increasing the residence time of cells in the upper reaches of the photic zone and (2) by increasing the rate at which nutrients are supplied to the photic zone. The settling velocities of phytoplankton cells range between 0 and 10 m day^{-1} (for a review see Smayda, 1970), with most values falling between 0.5 and 2 m day^{-1} (Eppley et al., 1967; Strickland et al., 1969). Average upwelling velocities are of the order of 10 m day^{-1} (Hidaka, 1954), which is quite sufficient to inhibit the sinking of negatively buoyant cells.

Since the netplankton fraction was primarily composed of nonmotile diatoms and the nannoplankton fraction of flagellates, it is probable that vertical water movements will have a greater effect on the vertical distribution of netplankton than on the nannoplankton. It is not surprising, therefore, that the depth of the netplankton maximum was more closely tied to the upward and downward trends of the isotherms, both seasonally at CalCOFI 3 (Figure 6) and along inshore-offshore transects of the California Current (Figure 8). The netplankton maximum at CalCOFI 3 was always found below that of the nannoplankton except during strong upwelling when both maxima occurred in the upper 10 m. During periods of subsidence the netplankton minimum was depressed to greater depths than the nannoplankton maximum was. This was observed during the Mixed Period even though NO_3-N concentrations in the surface layers were still high (>1.0 μM). The reverse was observed along transect 1-B in that the netplankton maximum decreased in depth as the zone of offshore upwelling was approached, moving in the process from a nitrate-rich layer (>5.0 μM NO_3-N) into a nitrate-poor layer (<0.5 μM NO_3-N). The depth distribution of nannoplankton chlorophyll (Figures 6 and 7) was more independent of vertical water movements and maximum chlorophyll concentrations were often found at the surface during influxes of oceanic water (during both Oceanic and Mixed Periods) when subsidence was most pronounced.

Most of these trends in the depth distribution of netplankton and nannoplankton chlorophyll could be explained in terms of the vertical distribution of nitrate in the photic zone. However, during the early stages of upwelling in

March, the netplankton maximum moved progressively toward the surface while the chlorophyll concentration of the maximum and in the water column steadily decreased. If this change in depth was due solely to the upward movement of the nitrate-rich layer in the photic zone, some increase in netplankton would have been observed during the time taken for the maximum to move from a depth of 75 m to 5 m. In addition, measurements made in the Peru Current, where vertical advection was not in evidence and the photic zone was well stratified (Malone, in press a), support the hypothesis that upward water movements, in addition to high nitrate concentrations, are necessary prerequisites for netplankton productivity to approach or exceed that of the nannoplankton. Netplankton productivity and the net/nanno productivity ratio were low despite high nitrate concentrations (Figure 13).

Two lines of evidence indicate that the netplankton and nannoplankton respond differently to varying nitrate concentrations. The first is based on the relationship between productivity and nitrate concentrations encountered in different environments (Figure 13). Nannoplankton productivity increased rapidly as NO_3-N increased from about 0.0 to 0.5 μM. Above 0.5 μM nannoplankton productivity increased asymptotically. In contrast, netplankton productivity increased slowly over concentrations of 0.0 to 1.5 μM and then increased rapidly with concentrations in excess of 1.5 μM (California Current system). The netplankton, therefore, tend to have higher half-saturation constants and maximum uptake rates for nitrate than the nannoplankton, so that NO_3-N concentrations above 1 to 3 μM are necessary before the netplankton can effectively compete with the nannoplankton. This agrees with the results of MacIsaac and Dugdale (1969) and Eppley et al. (1969), which indicate that small-celled oceanic species in oligotrophic waters have K_s values for nitrate uptake of less than 0.5 μM while large-celled neritic species in eutrophic waters have K_s values greater than 1.0 μM.

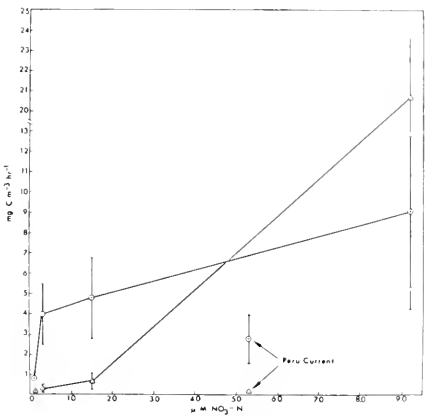


FIGURE 13.—Mean netplankton (△) and nannoplankton (○) productivity as a function of mean NO_3-N concentrations with 95% confidence limits: 0.1 μM, offshore oceanic region; 0.3 μM, CalCOFI 3, Oceanic Period; 1.5 μM, CalCOFI 3, Mixed Period; 9.2 μM, inshore upwelling.

The observed inshore vertical distributions of netplankton and nannoplankton chlorophyll were also consistent with these observations. The netplankton chlorophyll maximum was always found at depths where NO_3-N concentrations were greater than 2 μM, while during nonupwelling periods (when concentrations less than 2 μM were found in the photic zone) the nannoplankton maximum occurred at depths where the NO_3-N concentration was between 0.2 and 2.0 μM. Similar observations were made by Eppley (1970) who found that diatoms were associated with relatively high nitrate concentrations at depths where light intensities were high enough for growth to occur.

Based on these observations, netplankton productivity and standing crop will increase relative to the nannoplankton only when NO_3-N concentrations above 1 to 3 μM are found in the upper half of the photic zone and when the netplankton standing crop is supported in the photic zone by vertical advection, i.e., upwelling.

Decreases in netplankton standing crop and net nanno ratios were related to influxes of oceanic water and increases in grazing pressure in Monterey Bay. Variations in phytoplankton

productivity and grazing conformed to what Cushing (1959) has referred to as an unbalanced seasonal cycle of primary production and primary consumption. Neritic regions in temperate waters are generally characterized by about a 2-month time interval between peaks in phytoplankton and zooplankton biomass (Cushing, 1959; Heinrich, 1962), with a time lag of about 1 month between the onset of the spring bloom and the increase in zooplankton standing crop. Martin (1965) found a 2-month lag between the maximum phytoplankton standing crop and the increase in zooplankton standing stock.

In Monterey Bay, about 2 months elapsed between the March-April phytoplankton bloom and the rapid increase in grazing pressure observed during June and July (Figure 3). Although upwelling was in progress (NO_3-N concentrations were greater than 5 μM throughout the photic zone and the netplankton chlorophyll maximum was in the upper 10 m), the phytoplankton chlorophyll content of the water column declined as grazing pressure increased. The netplankton fraction fell continuously while the nannoplankton dropped at first and then increased (Figure 5). The reduction in standing crop was accompanied by a steady decline in the ratio of netplankton-to-nannoplankton chlorophyll in the water column, from 1.1 near the beginning of the increase in grazing pressure to 0.1 at its peak. Thus, it appears that (1) the phytoplankton bloom was ultimately limited by grazing; (2) the netplankton fraction, dominated by *Nitzschia* spp. and *Rhizosolenia* spp. (80% of the netplankton by number), was selectively grazed; and (3) the cycle of netplankton production and animal grazing was unbalanced.

Variations in the net nanno chlorophyll m^{-2} ratio were significantly related to concurrent changes in the nitrate content of the photic zone (an indicator of upwelling) and to grazing pressure ($F = 5.56$, $P = 0.05$) by the multiple regression equation:

$$\text{net nanno} = 1.76 + 0.003\ (NO_3\text{-N}) - 2.53\ (\text{Phaeo Chl-a}).$$

This equation is based on 20 sets of data (CalCOFI 3), and the partial correlation coefficients for the interactions between the net/nanno ratio and nitrate concentration ($r = +0.51$) and between the ratio and grazing pressure ($r = -0.56$) are significant at the 0.05 level. The evidence suggests, therefore, that upwelling is a necessary precondition for netplankton productivity and standing crop to approach or exceed that of the nannoplankton in marine environments where water depth greatly exceeds the maximum depth of wind-driven turbulent mixing.

The relative constancy of the nannoplankton relative to the netplankton fraction, in spite of marked changes in the concentration of inorganic nitrogen, the intensity and direction of vertical water movements, and grazing pressure, is puzzling. The assimilation ratios of both fractions exhibited little variability, but on the average nannoplankton ratios were twice as great as those of the netplankton. Since this ratio is an index of growth rate (cf. Eppley and Strickland, 1968), the nannoplankton must have been limited primarily by "cropping" factors (Dickman, 1969), at least during those periods when netplankton productivity was increasing relative to nannoplankton productivity. This is supported by the observation that the chlorophyll content of nannoplankton and netplankton cells also exhibited little variability during the period of study. During upwelling, two processes could selectively remove nannoplankton cells from upwelling regions: (1) grazing and (2) horizontal advection.

If nannoplankton grazers were predominantly protozoans (Beers and Stewart, 1969) with short generation times and netplankton grazers were crustaceans and fishes with long generation times, the coupling between primary productivity and grazing would be much closer for nannoplankton-based food chains than for netplankton-based food chains. The cycle of nannoplankton productivity and animal grazing would be balanced (Cushing, 1959; Heinrich, 1962) in contrast to the unbalanced character of netplankton-based food chains. This would tend to dampen fluctuations in the nannoplankton fraction relative to the netplankton fraction.

Similarly, if nannoplankton cells were selectively removed from sites of upwelling by mass

transport normal to the coast because of their lower sinking rates, netplankton cells would have a greater tendency to remain closer to the region of upward water movement than nannoplankton cells (Stommel, 1949). Both of these processes, selective grazing by organisms with short generation times and horizontal advection away from upwelling sites, would limit increases in nannoplankton standing crop during upwelling and could compensate for the growth rate differential between the netplankton and nannoplankton fractions. This would set the stage for netplankton productivity and standing crop to exceed that of the nannoplankton during upwelling, and also explain the discrepancy between nannoplankton growth rates and their response to photic zone enrichment. Decreases in nannoplankton standing crop due to "excessive" grazing or removal from the photic zone by downward water movements would be dampened by the short generation times (and, therefore, potentially rapid response time) and motility of the nannoplankton species.

Comparisons of the Oceanic Period in Monterey Bay with the offshore oceanic environment of the California Current reveals an interesting pattern of netplankton and nannoplankton variation which is consistent with the above model. The productivity and standing crop of the netplankton fraction did not vary significantly between the Oceanic Period inshore and the offshore oceanic zone. In contrast, the nannoplankton were significantly higher inshore than offshore (Table 5). This "inshore enhancement" effect during intrusions of oceanic water could arise in response to the overall pattern of circulation. The vertical distribution of nannoplankton chlorophyll compared with that of the netplankton indicated that nannoplankters are more independent of vertical water movements and are better able to maintain their position in the water column. This ability, probably a consequence of motility, will result in a concentration of nannoplankton in regions of downward flow (Hutchinson, 1967). In addition, the ability of nannoplankton to maintain their position in the photic zone could give rise to a situation analogous to the "island mass effect" described by Doty and Oguri (1956). The former is more likely, however, since assimilation ratios were equivalent in both inshore and offshore environments, i.e., the increase in primary productivity was a consequence of higher standing crops rather than an increase in growth rates.

TABLE 5.—Mean netplankton and nannoplankton productivity and standing crop with 95% confidence limits for the Oceanic Period at CalCOFI 3 and the offshore oceanic region of the California Current.

Oceanic region	Measurement	Nanno		Net	
Offshore	mgC m^{-3} hr^{-1}	0.69	± 0.20	0.08	± 0.05
Inshore		3.90	1.53	0.25	0.20
Offshore	mgChl-a m^{-3}	0.093	0.036	0.019	0.010
Inshore		0.477	0.227	0.068	0.047
Offshore	mgChl-a m^{-2}	15.60	3.22	2.58	0.92
Inshore		18.14	5.15	3.71	2.17

SUMMARY AND CONCLUSIONS

Phytoplankton productivity and standing crop were low under oceanic conditions, both inshore and offshore. During the Oceanic Period in Monterey Bay the nannoplankton accounted for 60 to 99% of the observed productivity and standing crop, while offshore this fraction was responsible for 75 to 99%. The productivity and standing crop of the netplankton fraction were exceedingly low and constant under these conditions, but the nannoplankton fraction was significantly higher inshore than offshore. Netplankton productivity and standing crop exceeded that of the nannoplankton only during periods of strong upwelling.

The netplankton fraction was composed almost exclusively of diatoms while the nannoplankton fraction was dominated by flagellates. Similar, but more detailed observations off La Jolla, Calif., (Reid et al., 1970) showed the nannoplankton to be composed primarily of naked dinoflagellates, "monads" (e.g., *Chilomonas marina* and *Eutreptia* sp.), and coccolithophores (e.g., *Coccolithus huxleyi*).

The nannoplankton fraction was surprisingly stable both seasonally in Monterey Bay and geographically in the California Current system. Variations in phytoplankton productivity and standing crop were due primarily to the netplankton with the nannoplankton maintaining a comparatively stable background level.

Increases in netplankton productivity and standing crop were closely related to upwelling, both as a consequence of the positive vertical advection and the entrainment of nitrate into the upper half of the photic zone. The requirement for positive vertical advection was probably related to both cell size and motility so that the vertical distribution of nannoplankters was more independent of vertical water movements. The relationship between cell size and A/V ratios probably accounted for the higher nitrate requirements of the netplankton. Decreases in the netplankton were primarily due to grazing and to removal from the photic zone by downward water movements.

The stability of the nannoplankton compared to the variability of the netplankton is interesting, especially in light of the marked changes observed in the concentration of inorganic nitrogen compounds and the direction and intensity of vertical water movements. Since nannoplankton assimilation ratios were consistently high and twice as great as netplankton assimilation ratios, the nannoplankton must have been limited primarily by cropping factors during upwelling periods when netplankton standing crop was increasing relative to that of the nannoplankton. Under these conditions increases in the nannoplankton fraction will be dampened by selective removal from upwelling sites by mass transport away from the coast and grazing by organisms with short generation times (e.g., protozoans). Decreases in nannoplankton standing crop due to "excessive" grazing or removal from the photic zone by downward water movements will be limited by the motility and short generation times of nannoplankton species. The motility of nannoplankters in combination with onshore mass transport and downward water movements will also favor an offshore-inshore increase in nannoplankton productivity and standing crop.

Finally, it is clear that the nannoplankton and netplankton components of phytoplankton communities respond differently to changes in their environment; that cell size, surface-to-volume ratios, and motility play important roles in mediating these responses; and that changes in netplankton and nannoplankton productivity relative to each other have definite consequences with respect to energy flow through phytoplankton-based food chains.

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DISTRIBUTION, APPARENT ABUNDANCE, AND LENGTH COMPOSITION OF JUVENILE ALBACORE, *Thunnus alalunga*, IN THE SOUTH PACIFIC OCEAN

HOWARD O. YOSHIDA[1]

ABSTRACT

The distribution, apparent abundance, and length composition of juvenile albacore, *Thunnus alalunga*, were deduced from 127 specimens found in the stomachs of 2,297 billfishes collected in the South Pacific between January 1964 and July 1966. Juvenile albacore were found in the South Pacific from lat 5° to 31° S, between long 153° and 179° W. Billfish stomach samples were collected from as far east as long 135° W, but juveniles were not in the stomachs east of long 153° W. The juveniles were consistently more numerous between lat 10° and 20° S than in the area farther north. The mean length of the juveniles increased from north to south but not from east to west (or vice versa). A southward migration of juveniles is postulated.

In April 1963, the National Marine Fisheries Service (formerly the Bureau of Commercial Fisheries), Hawaii Area Fishery Research Center established a field station in Pago Pago, American Samoa, to collect information on the longline fishery based there. A fleet of vessels from Japan, the Republic of Korea, and the Republic of China has supplied two American-owned canneries with albacore, *Thunnus alalunga*, and other tuna. In 1965 the fleet was composed of 154 vessels which landed 15,588 metric tons of albacore (Otsu and Sumida, 1968).

The field station was established primarily to study the effects of the fishery on albacore. As part of this study an investigation was started to determine the early life history of albacore in the South Pacific.

Beginning in 1964, arrangements were made with several longline fishing vessels based in American Samoa to collect stomachs of billfishes, which are known to prey on juvenile tunas (Yoshida, 1965, 1968). The distribution, apparent abundance, and length composition of juvenile albacore, here defined as fish smaller than 400 mm standard length, were determined by specimens found in the stomachs of the predators.

MATERIALS AND METHODS

The longline vessels based at American Samoa fished primarily for albacore; billfishes were taken only incidentally. The crews of the cooperating longline vessels collected 2,297 billfish[2] stomachs between January 1964 and July 1966. These stomachs were also used in a study of juvenile skipjack tuna, *Katsuwonus pelamis* (Yoshida, 1971).

In the laboratory, all the tunas and tunalike specimens were first sorted from the stomach contents. The juvenile tunas were identified by the use of skeletal characters; juvenile albacore were easily identified by their definitive skeletal characters (Matsumoto, 1963; Yoshida, 1965). Standard length (SL) was taken for all intact juveniles and is the measurement used throughout. For fragmentary specimens, the standard length was estimated from previously determined relations between standard length and various vertebral segments (Yoshida, 1968). A total of 127 juvenile albacore was found in the billfish stomachs (Table 1).

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[2] For the purpose of this paper the term billfish, in addition to the Istiophoridae, includes the swordfish, *Xiphias gladius*.

TABLE 1.—Juvenile albacore found in billfish stomachs from the South Pacific.

Date 1964	Position		Standard length	Date 1965	Position		Standard length	Date 1966	Position		Standard length
	Lat S	Long W			Lat S	Long W			Lat S	Long W	
			mm				mm				mm
3/9	07°00′	170°00′	143	1/5	14°00′	174°00′	122	1/7	15°00′	175°00′	107
3/20	06°00′	164°00′	116	1/5	14°00′	174°00′	97	1/9	15°00′	174°00′	89
3/21	05°00′	165°00′	67	1/22	14°00′	175°00′	96	1/9	15°00′	174°00′	83
4/4	08°00′	175°00′	102	1/22	14°00′	175°00′	78	1/9	16°00′	174°00′	110
4/5	08°00′	175°00′	136	1/22	14°00′	175°00′	84	1/9	16°00′	174°00′	113
4/9	07°00′	178°00′	78	1/24	14°00′	174°00′	102	1/9	16°00′	174°00′	133
4/9	07°00′	178°00′	137	1/25	14°00′	174°00′	109	1/17	12°00′	175°00′	106
4/12	07°00′	178°00′	26	1/26	11°00′	162°40′	81	1/23	12°40′	171°00′	133
4/16	07°00′	179°00′	57	1/27	14°00′	174°00′	118	2/8	09°00′	172°00′	130
4/19	07°00′	178°00′	102	1/29	14°00′	175°00′	110	2/8	09°00′	172°00′	137
4/20	07°00′	179°00′	66	1/30	14°00′	175°00′	102	2/8	09°00′	172°00′	130
4/20	07°00′	179°00′	66	4/4	07°10′	164°13′	123	2/8	09°00′	172°00′	93
4/20	07°00′	179°00′	106	4/12	07°10′	170°20′	102	2/13	07°02′	171°30′	102
4/24	09°00′	176°00′	115	4/22	07°00′	174°45′	86	3/7	06°35′	162°20′	58
4/25	08°00′	176°00′	67	4/22	07°00′	174°45′	86	3/14	07°20′	161°15′	89
4/26	08°00′	177°00′	119	9/1	13°00′	171°00′	96	3/16	08°10′	165°13′	67
5/8	07°54′	165°32′	118	11/7	16°30′	159°20′	152	3/22	11°32′	168°29′	104
5/8	07°54′	165°32′	113	11/7	16°30′	159°20′	158	3/22	11°32′	168°29′	116
5/8	07°54′	165°32′	104	11/7	16°30′	159°20′	142	3/28	09°10′	153°00′	168
5/8	07°54′	165°32′	113	11/8	16°20′	159°09′	152	5/5	08°13′	175°42′	83
5/8	07°54′	165°32′	88	11/22	17°00′	168°00′	105	5/5	08°13′	175°42′	92
5/8	07°54′	165°32′	106	11/29	17°07′	170°43′	119	5/5	08°13′	175°42′	97
5/8	07°54′	165°32′	125	11/29	17°07′	170°43′	115	5/7	08°00′	175°45′	94
5/10	07°46′	165°29′	121	11/29	17°07′	170°43′	91	5/9	08°28′	175°57′	53
5/10	07°46′	165°29′	88	12/14	17°50′	159°30′	137	6/8	07°15′	164°42′	74
5/19	05°29′	170°06′	48	12/14	17°50′	159°30′	148	6/29	30°49′	166°13′	314
5/19	06°00′	172°00′	47	12/17	16°11′	157°27′	100	7/12	28°50′	156°03′	297
5/23	06°00′	173°00′	58	12/21	17°52′	158°20′	138	7/15	28°05′	155°24′	317
5/26	06°23′	173°59′	99	12/22	10°00′	174°00′	159	7/23	28°44′	154°15′	321
6/1	05°52′	174°41′	48	12/25	18°00′	163°00′	130	7/23	28°44′	154°15′	316
6/1	05°52′	174°41′	73	12/25	18°00′	163°00′	164	7/24	28°22′	154°24′	301
6/20	06°43′	171°26′	53	12/28	17°30′	162°10′	162				
6/25	07°18′	172°53′	86	12/29	12°00′	171°00′	93				
8/18	26°00′	175°00′	357	12/30	12°00′	171°00′	115				
8/21	26°00′	176°00′	358	12/31	12°00′	171°00′	93				
9/4	24°00′	174°00′	328								
9/5	25°00′	174°00′	343								
9/9	25°00′	171°00′	333								
9/9	25°00′	171°00′	301								
9/13	18°00′	170°00′	230								
9/13	18°00′	170°00′	230								
9/13	18°00′	170°00′	223								
9/19	16°00′	173°00′	110								
10/4	09°00′	179°00′	93								
10/4	09°00′	179°00′	116								
10/4	15°00′	173°00′	93								
10/8	19°00′	173°00′	146								
10/9	17°00′	173°00′	185								
10/18	13°00′	174°00′	62								
10/22	15°00′	174°00′	83								
10/22	15°00′	174°00′	93								
10/22	15°00′	174°00′	78								
11/11	16°00′	171°00′	79								
11/13	16°00′	171°00′	62								
11/13	16°00′	171°00′	128								
11/14	16°00′	171°00′	133								
11/15	16°00′	175°00′	150								
11/16	16°00′	172°00′	110								
11/16	16°00′	172°00′	150								
12/18	14°00′	174°00′	102								
12/31	14°00′	175°00′	133								

LENGTH OF JUVENILES

The juvenile albacore in the billfish stomachs ranged from 26 to 358 mm (Figure 1). Two length groups were apparent in the length-frequency distribution: one with a mode at 110 mm and the other at 310 mm; another length group was suggested between 200 and 250 mm.

In the course of the study it became apparent that the larger juveniles were being taken between lat 20° and 30° S. To determine if differences existed in juvenile sizes by latitude, lengths were plotted against latitude of capture (Figure 2). The smallest individuals were taken north of lat 10° S and the largest south of lat 20° S. No specimens smaller than 290 mm were taken south of lat 20° S. The juveniles between lat 10° and 20° S were slightly larger (mean SL 120 mm) than those taken north of lat 10° S (mean SL 94 mm). The mean standard length of those from south of lat 20° S was 324 mm.

In contrast to the latitudinal differences in juvenile length, no longitudinal trends in length were evident. Juveniles larger than 300 mm were taken in the eastern as well as the more westerly portion of the area sampled.

MIGRATION OF JUVENILES

The differences in the lengths of the albacore in the three latitudinal bands may be caused by the migration of the juveniles. The increase in length of juveniles from north to south and the absence of any longitudinal trends in length suggest a southward migration. After attaining a length of nearly 200 mm the juveniles that originate between the equator and lat 10° S probably start migrating south. They apparently continue to move southward as they grow. This migration would explain the absence of large (>250 mm) juveniles north of lat 20° S.

These observations on the suspected migration pattern of juvenile albacore fit well with the accumulated information on the biology of albacore in the South Pacific. Observations on the length composition of commercial catches of albacore in the South Pacific indicate latitudinal differences in the size of albacore. Adult albacore tend to be small north of lat 15° S and

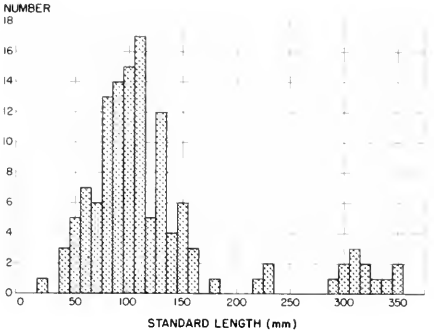

FIGURE 1.—Length-frequency distribution of juvenile albacore in the South Pacific.

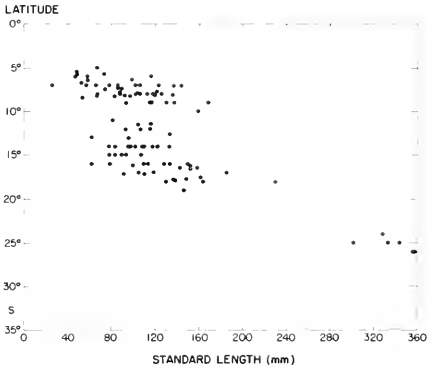

FIGURE 2.—The length of juvenile albacore plotted against latitude of capture.

largest between lat 20° and 25° S. They are smaller again south of lat 25° S (Otsu and Sumida, 1968). Length-frequency data published by the Nankai Regional Fisheries Research Laboratory (1959) show that albacore as small as 490 to 500 mm are caught on longlines south of lat 30° S. Thus, it could be that the juveniles move southward as they grow and are first taken by the longline fishery as preadults in the higher latitudes in the South Pacific. A similar pat-

tern has been deduced for albacore in the North Pacific. Otsu and Uchida (1963) hypothesized that the juveniles in the North Pacific migrate from tropical and subtropical waters into temperate waters and are recruited into the adult population in higher latitudes, presumably north of lat 30° N. Generally speaking, then, the albacore in the North and South Pacific, which are believed to constitute separate subpopulations, follow similar migration patterns within their respective hemispheres. The adults are believed to spawn in lower latitudes, between the equator and lat 20° where the eggs hatch and the larvae develop into juveniles. The juveniles then migrate into higher latitudes as they grow and they join the adult population in the higher latitudes.

AGE AND GROWTH

Information on age and growth is useful in determining certain vital statistics for fish populations and it would be useful if the growth of juvenile albacore in the South Pacific could be determined. Around Hawaii juveniles (60-350 mm SL) were estimated to grow about 31 mm per month (Yoshida, 1968). It would have been interesting to compare this growth with that of juveniles in the South Pacific. A plot of juvenile albacore length by time of capture, however, did not indicate that the length of the juveniles was increasing with time.

DISTRIBUTION AND ABUNDANCE

Because the data for any one year were sparse, the data for all years were combined to determine the quarterly distribution of juvenile albacore (Figure 3). The apparent distribution of juvenile albacore may reflect the operations of the longline boats. The longline vessels based at American Samoa primarily were seeking albacore and selecting areas where albacore catch rates tended to be high. The vessels generally fished north of lat 20° S in the first half of the year and beginning in June or July moved southward to as far south as lat 30° S (Otsu and Sumida, 1968). The data indicated that the cooperating vessels generally followed this pattern. In spite of this shortcoming the data suggest some interesting features of the seasonal distribution of juvenile albacore.

In the first quarter billfish stomach samples were collected between long 150° and 178° W and lat 5° and 16° S. The juveniles were generally found throughout the sampling area west of long 153° W.

In the second quarter the longitudinal range of sampling was slightly greater but the juveniles were restricted to the west between long 165° and 179° W. Latitudinally, most of the stomach samples were from north of lat 10° S except for a few samples from about lat 31° S. Juveniles were taken between lat 5° and 9° S and at lat 31° S.

In the third quarter stomach samples were available from long 140° W to 178° E between lat 5° and 31° S. The juveniles were absent in samples from north of 14° S.

In the fourth quarter the sampling area was bounded by long 135° W and 178° E and lat 6° and 21° S. Juveniles were absent from the easternmost portion of the area. They were taken between long 157° and 179° W and lat 9° and 20° S. These observations suggest that the center of the spawning area is closer to the area between long 160° W and the 180th meridian than farther to the east.

Variations were also evident in the apparent abundance of juvenile albacore as indicated by the number of juveniles found monthly per 100 billfish stomachs (Figure 4).

Juvenile albacore were found in all months except July and August. Peaks in apparent abundance occurred in January, April, and November.

The data for all years were pooled because billfish stomachs were unavailable for some months in some years. Also, estimates of apparent abundance may be biased by the small sample sizes. The shortcoming of combining data for all years and considering a large area as an entity is that annual and areal variations in apparent abundance are obscured. For example, in 1965, except in April when four juvenile albacore were taken, no juveniles were found in billfish stomachs from north of lat 10° S.

The overall abundance of juveniles was greater

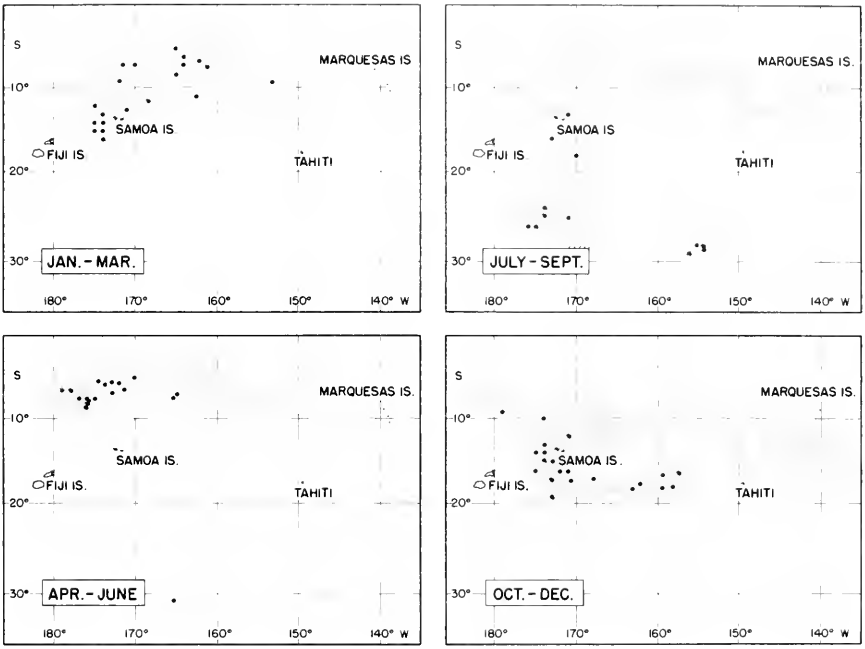

FIGURE 3.—The quarterly distribution of juvenile albacore in the South Pacific, all years combined. The shadings show areas from which billfish stomachs were collected. The dots show where juvenile albacore were taken.

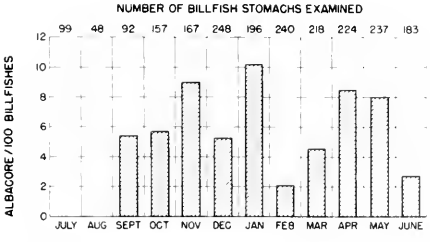

FIGURE 4.—Apparent abundance of juvenile albacore in the South Pacific.

between lat 10° and 20° S than in the area north of lat 10° S. Combining the data for all years, 7.5 juveniles per 100 billfishes were taken between lat 10° and 20° S, and 4.5 juveniles per 100 billfishes were taken north of lat 10° S. (Near Hawaii between July 1962 and April 1966, juvenile albacore were taken at a rate of 0.8 per 100 billfishes [Yoshida, 1968]. Thus, juvenile albacore are apparently more numerous in the South Pacific than around Hawaii.)

SPAWNING

Variations in seasonal and geographic distribution and apparent abundance of juvenile albacore may be related to the spawning of the

adults. Several investigators have made observations on the spawning of albacore in the South Pacific. On the basis of an examination of ovaries, Otsu and Hansen (1962) concluded that peak albacore spawning occurs in the southern hemisphere summer between the equator and lat 20° S. Their results also suggest a spawning season of at least 5 months. Ueyanagi (1969), who based his study on the distribution of larvae and on the stage of maturity of ovaries of adults, also suggested a southern summer spawning season. The seasonal apparent abundance of juvenile albacore does not disagree with the above conclusions. The fact that juveniles were found in all but 2 months of the year suggests a long spawning season. The presence of juvenile albacore during and beyond the southern hemisphere summer indicates some spawning during the summer. My data also suggest annual variations in adult spawning. The virtual absence of juvenile albacore north of lat 10° S in 1965 indicates little or no spawning in this area during that year.

COMPARISON WITH SKIPJACK TUNA

A comparison of the distribution and abundance of juvenile albacore and skipjack tuna offers some interesting contrasts. Although billfish stomachs were sampled from long 135° W to 177° E, juvenile albacore were found only between long 153° and 179° W (Figure 5). The juveniles were found throughout the latitudinal range of sampling between lat 5° and 31° S. Juvenile skipjack tuna are distributed over a wider area: between long 137° W and the 180th meridian from lat 5° to 32° S (Yoshida, 1971). These distributional patterns indicate that skipjack tuna spawn over a wider area than the albacore in the South Pacific.

Comparing the abundance of the two species, in 1964 juvenile skipjack tuna were more numerous north of lat 10° S than from 10° to 20° S, but in 1965 they were more numerous between 10° and 20° S than in the area to the north (Yoshida, 1971). Juvenile albacore were consistently more abundant between lat 10° and 20° S than to the north. Also, juvenile skipjack tuna apparently were from 4.5 to 15 times more numerous than juvenile albacore between the equator and lat 20° S (Table 2). It is also interesting that the apparent abundance of both species declined from 1964 to 1965.

TABLE 2.—Apparent abundance of juvenile albacore and skipjack tuna in the South Pacific. The apparent abundance is expressed as number per 100 billfishes. Data for juvenile skipjack tuna are from Yoshida (1971).

Year	North of lat 10° S		Lat 10°-20° S	
	Albacore	Skipjack tuna	Albacore	Skipjack tuna
1964	6.7	46.3	9.3	42.6
1965	1.9	28.8	5.8	35.6

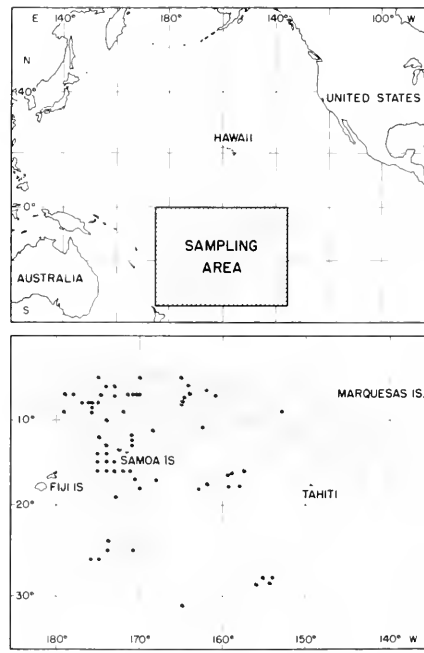

FIGURE 5.—The distribution of billfishes (shading) sampled by the cooperating longline vessels and the distribution of juvenile albacore (dots) found in the billfish stomachs.

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COMPARISON OF PHYTOPLANKTON PRODUCTION BETWEEN NATURAL AND ALTERED AREAS IN WEST BAY, TEXAS[1]

JANE CORLISS AND LEE TRENT[2]

ABSTRACT

Phytoplankton production was compared between an undredged marsh area, a bay area, and an adjacent marsh area altered by channelization, bulkheading, and filling. Average gross production (mg carbon/liter/day) in the altered area (canals) was 8% higher than in the marsh and 48% higher than in the bay during June, July, and August 1969. Gross and net production were significantly higher in the canals and marsh than in the bay; differences between the canals and marsh were not significant.

Large areas of shallow bays and marshes are being dredged, bulkheaded, and filled for waterfront housing sites along the Gulf of Mexico coast. When these sites are developed, shallow marsh and bay areas are deepened or filled with spoil, thus changing the environment for marine organisms. Major changes to the bayshore environment as a result of these alterations include: (1) reduction in acreage of natural shore zone and marsh vegetation; (2) changes in marsh drainage patterns and nutrient inputs; and (3) changes in water depth and substrates. The effects of these environmental changes on the productivity of estuarine organisms are poorly understood.

Basic production in estuaries results from four types of plant life: phytoplankton, attached algae, sea grasses, and emergent vegetation. Production of sea grasses and emergent vegetation is reduced or lost when natural marsh areas are dredged and filled for housing sites. Whether or not this reduction in primary production by sea grasses and emergent vegetation is compensated for by an increase in production by phytoplankton and attached algae is not clear. The objective of this study was to compare phytoplankton production between housing development canals, natural marsh areas, and the open bay in a shallow Texas estuary.

STUDY AREA AND METHODS

The study area in West Bay, Texas, included a natural marsh, an open bay area, and the canals of a waterfront housing development (Figure 1). The developed area, which included about 45 hectares of emergent marsh vegetation, intertidal mud flats, and subtidal water area prior to alteration, was reduced to about 32 hectares of subtidal water by dredging and filling. The water volume (mean low tide level) was increased from about 184,000 to about 394,000 kliter.

Sampling stations were established in dead-end canals in a housing development, natural marsh areas, and an open bay area (Figure 1). Water depths at mean low tide at stations 1 through 5 were 1.6, 2.6, 0.5, 0.2, and 1.0 m respectively.

Primary production was measured on six occasions at each station between June 18 and August 14, 1969. Measurements were made using the light- and dark-bottle technique designed by Gaarder and Gran (1927). Water samples were taken 15 cm below the surface at all stations. A 4-liter bottle having a vent at the bottom with a 30-cm rubber tube attached was used to take the subsurface samples. Number 10 netting (0.060-mm mesh) was placed over the mouth of the bottle and the bottle was submerged, mouth down, until the container filled. The netting was used to eliminate most of the zooplankton from the samples.

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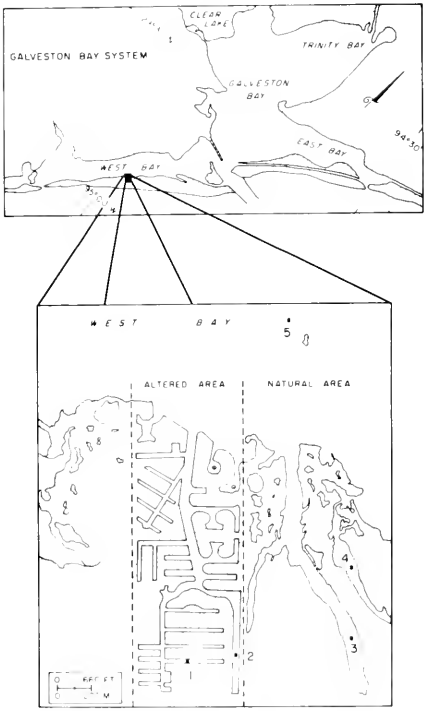

FIGURE 1.—Study area and sampling locations in the Jamaica Beach area of West Bay, Tex.

For each station, six biological oxygen demand (BOD) bottles (300 ml)—two wrapped with black rubber tape and four unwrapped—were filled (gravity flow) from the 4-liter water sample by inserting the rubber tube down to the bottom of each bottle. About 300 ml of water was permitted to overflow after the bottle was full. Two of the unwrapped bottle samples were fixed immediately for oxygen determination. The remaining bottles were stoppered and suspended 15 cm below the surface. The time of sampling was recorded for each station and the bottles were recovered 24 hr later and fixed for oxygen determination.

Water temperature (° C) and turbidity in Jackson turbidity unit—JTU (American Public Health Association, 1962)—observations were made just before the water samples for plankton were taken (Table 1); insolation was measured with a recording pyrheliometer located at station 1.

TABLE 1.—Water temperatures and turbidities observed just before each incubation period.

Date	Station					Average
	1	2	3	4	5	
				°C		
Temperature						
June 18	29.0	29.0	29.0	29.0	29.0	29.0
June 25	31.5	31.0	30.5	30.5	31.0	30.9
July 9	31.5	31.5	31.0	31.0	31.5	31.3
July 24	30.5	29.5	30.0	29.5	29.0	29.7
July 30	32.0	31.0	31.5	31.5	31.0	31.4
Aug. 13	31.5	31.0	30.5	30.5	32.0	31.1
Average	31.0	30.5	30.4	30.0	30.6	30.6
			Jackson turbidity units			
Turbidity						
June 18	9.0	8.0	16.0	16.0	12.0	12.2
June 25	11.5	13.0	29.0	24.5	53.0	26.2
July 9	9.5	8.0	18.0	24.0	14.0	14.7
July 24	12.5	10.0	29.5	24.5	27.0	20.7
July 30	9.0	8.5	21.0	18.5	17.0	14.8
Aug. 13	9.5	6.5	19.0	12.0	18.5	13.1
Average	10.2	9.0	22.1	19.9	23.6	16.9

Dissolved oxygen was measured using a modified Winkler method (Carritt and Carpenter, 1966). Oxygen determinations were made within 3 hr after fixing the water samples. Changes in dissolved oxygen were converted to changes in organic carbon using the relation formulated by Ryther (1956): 1.0 mg oxygen is equivalent to 0.30 mg carbon.

Net production (NP), respiration (R), and gross production (GP) were determined using the carbon values from the initial (I), light (L), and dark (D) bottle values as follows:

$$NP = L - I,\ R = I - D,\ \text{and}\ GP = NP + R.$$

ENVIRONMENTAL AND HYDROLOGICAL DATA

Surface water temperature varied no more than 1.5° C between stations on any sampling date and no more than 3° C between dates at

any station (Table 1). Surface water temperatures were slightly higher in the canals and bay than in the marsh.

Turbidity values of surface water samples varied as much as 41.5 JTU between stations on June 25 and as much as 41 JTU between dates at station 5 (Table 1). Average turbidity values from the marsh and bay stations were about double those from the canal stations. On June 25, however, turbidities in the bay were about twice those in the marsh and about four times those in the canals.

Insolation was similar on all sampling dates. The daily averages ranged from 0.82 to 0.85 cal/cm²/day.

Overproduction of phytoplankton, in terms of oxygen balance, occurred in some canals of the development. Plankton blooms that reduced oxygen to zero at night, and caused fish kills at station 1, occurred at least three times during the study period. These blooms were observed on July 4, July 18, and August 7.

PRODUCTION AND RESPIRATION

Average gross production ranged from 1.17 at station 5 to 2.25 mg carbon/liter/day at station 1 during the study (Table 2 and Figure 2). Average values at the two canal stations were almost identical. Likewise, there was almost no difference between average values at the two marsh stations. Average production in the canals was slightly higher (8%) than in the marsh and much higher (48%) than in the bay. In similar studies in Boca Ciega Bay, Fla., Taylor and Saloman (1968) reported that primary production of phytoplankton did not differ consistently between development canals and open bay areas.

Average net production ranged from 0.84 at station 5 to 1.74 mg carbon/liter/day at station 1. Like gross production, the values were about the same among canal stations and among marsh stations. Average net production in the canals was 13% higher than in the marsh and 51% higher than in the Bay.

Respiration averaged 0.51 mg carbon/liter/day, or 27.7% of gross production and ranged from 23.4 to 34.4% between stations (Table 2).

TABLE 2.—Net production (NP), respiration (R), gross production (GP), and percent respiration ($\%R$) by station and date in West Bay, Tex.

Date	Variable	Station 1	2	3	4	5	Average
		— mg carbon/liter/day —					
June 18	NP	2.01	0.91	0.70	1.08	0.54	1.05
	R	0.23	0.69	0.58	0.31	0.34	0.43
	GP	2.24	1.60	1.28	1.39	0.88	1.48
	$\%R$	10.3	43.1	45.3	22.3	38.6	31.9
June 25	NP	1.33	1.75	0.87	1.04	0.74	1.15
	R	0.39	1.07	0.63	0.52	0.37	0.60
	GP	1.72	2.82	1.50	1.56	1.11	1.74
	$\%R$	22.7	37.9	42.0	33.3	33.3	33.8
July 9	NP	1.80	1.08	1.61	1.34	0.84	1.33
	R	0.37	0.56	0.53	0.43	0.31	0.44
	GP	2.17	1.64	2.14	1.77	1.15	1.77
	$\%R$	17.0	34.1	24.8	24.3	26.9	25.4
July 24	NP	2.57	3.04	1.77	2.40	0.94	2.14
	R	0.43	0.38	0.44	0.70	0.32	0.45
	GP	3.00	3.42	2.21	3.10	1.26	2.60
	$\%R$	14.3	11.1	19.9	22.6	25.4	18.7
July 30	NP	0.81	1.30	1.82	2.12	0.74	1.36
	R	0.74	0.46	1.19	0.40	0.32	0.62
	GP	1.55	1.76	3.01	2.52	1.06	1.98
	$\%R$	47.7	26.1	39.5	15.9	30.2	31.9
Aug. 13	NP	1.94	1.90	1.43	1.57	1.26	1.62
	R	0.87	0.33	0.77	0.44	0.31	0.54
	GP	2.81	2.23	2.20	2.01	1.57	2.16
	$\%R$	30.9	14.8	35.0	21.9	19.7	24.5
Average	NP	1.74	1.66	1.37	1.59	0.84	1.44
	R	0.50	0.58	0.69	0.47	0.33	0.51
	GP	2.25	2.24	2.06	2.06	1.17	1.95
	$\%R$	23.8	27.8	34.4	23.4	29.0	27.7

Distinct differences in the percents of gross production attributable to respiration between canal, marsh, and bay areas were not apparent.

Averages of gross and net production in the canals and marsh were significantly greater than in the bay; differences between the canals and marsh were not significant (Table 3).

The differences in production between stations were related to turbidity. The correlation coefficient (r) between average gross production and average turbidity at each station was —0.70.

TABLE 3.—Comparisons of net productivity, respiration, and gross productivity between stations (one-way analysis of variance).

	Comparison between			
	Stations 1-5		Stations 1-4	
	df	F-value[1]	df	F-value
Net production	4,25	4.06*	3,20	0.72
Respiration	4,25	2.30	3,20	0.97
Gross production	4,25	5.05**	3,20	0.25

[1] Significance level:
* 5%
** 1%

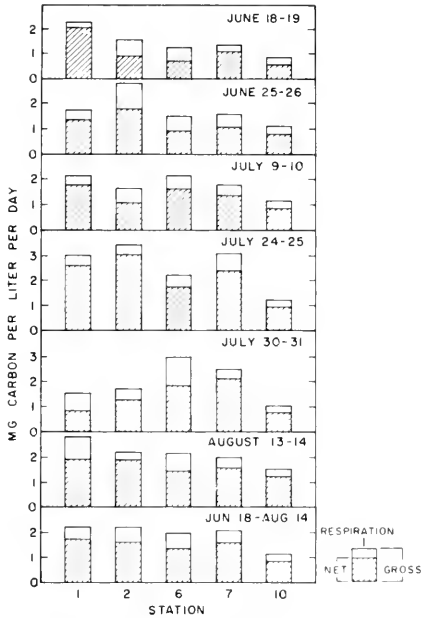

FIGURE 2.—Gross and net production and respiration by station and date, and average values for all sampling dates.

DISCUSSION

It is probable that eutrophic conditions will develop more frequently in housing development canals than in natural marsh areas because of differences in phytoplankton production, water circulation, water exchange, and high nutrient levels. In this study, gross production of phytoplankton in surface waters was higher in the canals than in the marsh or bay. We did not obtain information for computing production per unit area, but it is probable that production per unit area was significantly greater in the canals than in the other two areas, the reasons being the greater depths and lower turbidities in the canals. Wind-driven circulation responsible for reaeration of the waters in the development is less than in the natural area because of houses blocking and diverting prevailing winds and because many of the canals are narrow and perpendicular to the direction of prevailing summer winds. Water depths at mean low tide in the development averaged about 1.5 m but were often much greater, sometimes over 3 m, whereas depths in the natural area averaged about 0.6 m but were always less than 1 m. With the average tide level change of 0.3 m, this means that only about one-fifth of the volume of water in the development exchanges with the bay during a normal tidal cycle, whereas about one-half exchanges per cycle in the natural area. Nutrient levels were about the same (nitrogen) or slightly higher (phosphates) in the canals than in the natural area (Moore and Trent, 1971). It is possible, however, that because of reduced water exchange, nutrient levels in parts of the development were too high to maintain a balanced ecological system.

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THERMAL TOLERANCE OF JUVENILE PACIFIC SALMON AND STEELHEAD TROUT IN RELATION TO SUPERSATURATION OF NITROGEN GAS

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ABSTRACT

Thermal tolerance of juvenile chinook salmon (*Oncorhynchus tshawytscha*), coho salmon (*O. kisutch*), and steelhead trout (*Salmo gairdneri*) that had been held at various acclimation temperatures was lowered when test water was supersaturated (125-130% of saturation) with nitrogen gas. Increasing the depth of the test tank allowed the fish to compensate somewhat for the supersaturation by sounding, but substantial mortalities still occurred. A comparison of tolerance among the species tested revealed that coho salmon were the most tolerant, chinook salmon next, and steelhead trout the least tolerant to temperature increases in the presence of supersaturation of nitrogen.

During the past several decades, a number of investigators have examined temperature as a lethal factor by use of the classic pharmacological assay method. Fry, Hart, and Walker (1946) recognized the importance of acclimation temperature in determining the tolerance of a fish to high and low temperatures and established upper and lower levels of tolerance at various acclimation temperatures. Brett (1952) listed maximum temperatures for survival of young Pacific salmon (*Oncorhynchus* spp.) between 23.8 and 25.1° C. In later work (Brett, 1958), he emphasized temperatures below those at which a fish dies and constructed hypothetical temperature polygons which described lower levels of temperature tolerance where activity, growth, and spawning would be affected.

More recently, investigators have emphasized the response of fish to temperature changes under multivariate conditions. Many factors such as dissolved oxygen deficits, carbon dioxide increases, and increases in toxic substances all affect an aquatic organism's tolerance to temperature increases. Mihursky and Kennedy (1967) stressed the importance of multivariate experiments for establishing more realistic standards for temperature regulation.

Several nuclear power plants have been proposed for the Columbia River. The National Marine Fisheries Service (NMFS) is particularly concerned about the effect that heated effluents from these plants might have on juvenile Pacific salmon and steelhead trout (*Salmo gairdneri*) migrating downstream, particularly while they are stressed by supersaturated nitrogen gas. High levels of nitrogen gas (over 125% saturation) occur within large areas of the Columbia from about early May until mid-August (Ebel, 1969). This period coincides with the downstream migration of most juvenile salmon and trout. Although the effect of supersaturation of gas on juvenile salmon and trout has not been examined in great detail, preliminary studies by Ebel (1969) clearly show that Columbia River juvenile salmon have considerably lower tolerance to temperature increases when stressed by supersaturation of nitrogen than the tolerance indicated by Brett (1952).

The Federal Water Quality Administration (FWQA) recognized that supersaturation of dissolved nitrogen could be a significant factor in establishing water quality criteria for the Columbia River. It therefore contracted the Bureau of Commercial Fisheries (BCF; presently designated as NMFS) to determine the changes in tolerance of juvenile salmon and trout to temperature increases at different levels

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of temperature acclimation and supersaturation of nitrogen. In the experiments described in this report, BCF personnel sought to determine the change in tolerance of juvenile salmon and trout to temperature increases when stressed by supersaturation of nitrogen and to determine possible changes in tolerance if they had the option to sound to different depths. Ebel (1969) reported that the depth at which fish migrate influences the effect of supersaturation of nitrogen because the gas remains in solution at a much higher concentration when under pressure; we therefore considered depth as well as temperature increase and supersaturation of nitrogen to be important.

Our first series of experiments describe the effect of supersaturation of nitrogen and temperature increases at surface pressures. Later experiments show how depth changes the above effect.

METHODS

The general approach used to determine the effect of nitrogen supersaturation on the tolerance of juvenile salmon and trout to increased temperature was similar to that used by Brett (1952). Stocks were acclimated to temperatures identical to those used by Brett; test temperatures encompassed the ranges Brett used in his lethal temperature determinations. These test and acclimation temperatures were purposely selected so that changes in tolerance caused by the stress of supersaturated nitrogen could be compared with Brett's well-established levels of temperature tolerance.

Groups of 20 test fish, each acclimated to a given temperature, were placed simultaneously in control and test situations involving identical test temperatures at treatments of high (125-130%) and normal (100%) nitrogen saturation. Two acclimation groups were used in each set of tests—one that had a normal acclimation history and one that had been exposed to supersaturated nitrogen for 720 min. Observations of behavior and mortality were made continuously for the first 6 hr, then every hour for the remainder of an 18-hr period. Events recorded were times to first indication of stress, to loss of equilibrium, and to death. The numbers of live and dead fish with obvious external symptoms of gas bubble disease were recorded at the termination of tests. All tests were then repeated (most tests were duplicated, some were done three times); the data given in this report are derived from the average value of the duplicated tests.

Hatchery and wild stocks of fish were tested. Hatchery fish were from the following stocks: coho salmon (*O. kisutch*) reared at Leavenworth National Fish Hatchery, Leavenworth, Wash.; spring chinook salmon (*O. tshawytscha*) reared at Little White Salmon National Fish Hatchery, White Salmon, Wash.; and steelhead trout reared at the Washington State Fish Hatchery at Green River, Cumberland, Wash. The wild fish (spring chinook salmon) were collected from the turbine intake gatewells at McNary Dam on the Columbia River. Because of time limitations and lack of sufficient populations of fish, only the coho were tested through the entire range of acclimation temperatures (5, 10, 15, and 20° C). Hatchery-reared steelhead trout and hatchery and wild spring chinook were tested only after exposure at selected acclimation temperatures—steelhead at acclimation temperatures of 10° and 15° C and wild chinook at 10° C.

The experimental temperatures (test and acclimation), lengths of time that the various groups of fish were in holding and acclimation tanks, and size of fish at time of testing are summarized in Table 1. Water in test tanks was adjusted to the appropriate test temperatures established for each acclimation temperature. Temperatures were maintained within ±0.2° C in both test and acclimation tanks. When the test series required stressing of the acclimated fish with supersaturated nitrogen, an acclimation tank at the appropriate temperature was saturated at 115 to 120% nitrogen and about 114 to 120% oxygen. The fish were then transferred from the normally saturated tank (100%) to the supersaturated tank and stressed for 720 min. When supersaturated water was needed in the test tanks, the supersaturating equipment was activated, and each tank was adjusted to maintain between 125 and 130% nitrogen. To ensure stability, the temperatures and satura-

TABLE 1.—Holding time and temperature of water before transfer to acclimation tanks, acclimation conditions, and mean size at time of testing of three species of salmon and trout.

Species and origin of fish	Time in holding tank	Water temperature in holding tank	Acclimation conditions		Mean size of fish at time of test	
			Days	Temperature	Length	Weight
	days	*° C*		*° C*	*mm*	*g*
Hatchery coho	28	8.8	35	5.0a	117	17
	28	8.8	17	10.0a	118	18
	28	8.8	23	10.0	134	22
	28	8.8	4	12.5	134	22
	28	8.8	48	15.0a	134	22
	28	8.8	3	11.7	118	18
	28	8.8	4	14.0	118	18
	28	8.8	16	15.0	118	18
	28	8.8	4	17.5	118	18
	28	8.8	3	20.0a	118	18
Wild spring chinook	0	15.0	4	10.0a	129	19
Hatchery spring chinook	90	8.8-13.0	4	15.0a	134	23
	90	8.8-13.0	3	15.0	134	23
	90	8.8-13.0	3	17.0	134	23
	90	8.8-13.0	3	20.0a	134	23
Hatchery steelhead	10	12.0	5	10.0a	179	54
	10	12.0	3	14.0	179	54
	10	12.0	42	15.0a	179	54

a Final acclimation temperature.

tion levels were then measured for 24 hr before introduction of the test fish.

The source of supersaturation of nitrogen gas in the Columbia River was entrained air. We used pumps to entrain air in our test and acclimation tanks. Oxygen, therefore, was also supersaturated; the ratio of oxygen to nitrogen supersaturation was similar to that recorded in the river (Beiningen and Ebel, 1971). Oxygen saturation varied from about 114 to 120%.

Facilities used for tolerance tests in relation to supersaturation of nitrogen at surface pressures were designed to provide a continuous supply of fresh water. Sufficient cooling and heating capacity was available from a water heater and chiller to continuously supply 227 liters/min of either heated or chilled water from 5° to 40° C. Ten cylindrical fiber glass acclimation tanks, about 1 m high × 2½ m in diameter, provided sufficient space to maintain 2000 fish at each of five acclimation temperatures without crowding. The test tanks were rectangular fiber glass tanks of 113.6-liter capacity. A flow of approximately 11.5 to 30.0 liters/min was maintained in acclimation tanks and 3.5 liters/min in test tanks.

Supersaturation of nitrogen and oxygen was achieved in the acclimation tanks by metering air into the intake of two high-pressure recirculating pumps with 42 kg/cm² back pressure on the discharge side of the pump. About 0.75 liter/min air in each pump created the desired saturation of nitrogen (115-120%). Supersaturation of nitrogen and oxygen in the test tanks was achieved by metering 0.05 liter/min into another high-pressure recirculating pump with 32 kg/cm² back pressure, which recirculated the water in the coldwater supply tank to the test tanks. (Source of water was from Seattle municipal supply; chlorine was eliminated by charcoal filters.) The final saturation value for each tank was then achieved by manipulating the number of equilibrating screens through which the water flowed before entering the tanks.

The test tank (9 m deep) used for our experiments concerning water depth has been described by Pugh, Groves, and Ebel (1969). When tolerance tests were conducted in the deep tank, the saturation levels of nitrogen and oxygen were controlled in the above manner (by injecting air into a recirculating pump). Because of the large volume of water (66,648 liters), a continuous flow of fresh water was not needed and the existing water was recirculated. Time limitations precluded testing more than two populations (hatchery coho and wild spring

chinook salmon) at 10° C acclimation temperature.

EFFECT OF SUPERSATURATION OF NITROGEN GAS ON TOLERANCE OF FISH TO TEMPERATURE INCREASES

Supersaturation of nitrogen (125-130%) in the test tanks lowered the tolerance of all fish to temperature increases at all acclimation temperatures when tested below 26° C (Tables 2-10). More than 50% mortality occurred within 18 hr even with no temperature increase when the test tanks were supersaturated at this level. Time to death of fish was accelerated regardless of acclimation temperature.

A comparison of LE_{50} (exposure time when 50% of the population is dead) curves developed under the four treatment levels indicates that a prior stress of 115 to 120% saturation for 12 hr did not greatly affect the fish when they were subjected to temperature increases in water saturated at 100% (Figure 1). When these

TABLE 3.—Mean values[a] of lethal exposure time (in minutes) for coho salmon that had been acclimated to 10° C water temperature and then subjected to various increases in water temperature and saturation of nitrogen gas.

Dead fish in sample	Level of gas[b]	Lethal exposure time at various temperatures (° C)					
		10	15	20	23	25	27
One fish	NN	--	--	--	--	130	4
	NS	773	413	360	215	130	6
	SN	--	--	--	--	135	4
	SS	770	407	340	155	125	12
Three fish	NN	--	--	--	--	100	< 5
	NS	877	472	517	297	152	16
	SN	--	--	--	--	67	7.5
	SS	960	480	382	225	95	15
Half of sample	NN	--	--	--	--	167	15
	NS	--	1,085	540	550	192	23
	SN	--	--	--	--	232.5	16.5
	SS	960	480	480	282.5	193.5	35.5
All of sample	NN	--	--	--	--	215	32.5
	NS	--	--	--	--	255	72.5
	SN	--	--	--	--	255	35
	SS	--	--	1,050	750	395	70

[a] Mean of replicated tests.
[b] NN = normal saturation (100%) of nitrogen gas in acclimation and test tanks;
NS = normal saturation in acclimation tank and supersaturation (125-130%) in test tanks;
SN = supersaturation in acclimation tank and normal saturation in test tanks;
SS = supersaturation in acclimation and test tanks.

TABLE 2.—Mean values[a] of lethal exposure time (in minutes) for coho salmon that had been acclimated to 5° C water temperature and then subjected to various increases in water temperature and saturation of nitrogen gas.

Dead fish in sample	Level of gas[b]	Lethal exposure time at various temperatures (° C)					
		5	10	15	20	23	25
One fish	NN	--	--	--	--	125	15.5
	NS	525	412	380	230	85	14
	SN	--	--	--	--	102	8
	SS	250	330	197	112	35	10
Three fish	NN	--	--	--	--	167	20.5
	NS	615	457	405	270	108	16.5
	SN	--	--	--	--	270	12
	SS	483	435	315	128	137	13
Half of sample	NN	--	--	--	--	300	27
	NS	810	605	577	390	266	21.5
	SN	--	--	--	--	--	20
	SS	626	580	435	229	186	20
All of sample	NN	--	--	--	--	--	64
	NS	--	990	--	1,080	420	58
	SN	--	--	--	--	--	60
	SS	--	--	--	840	450	445

[a] Mean of replicated tests.
[b] NN = normal saturation (100%) of nitrogen gas in acclimation and test tanks;
NS = normal saturation in acclimation tank and supersaturation (125-130%) in test tanks;
SN = supersaturation in acclimation tank and normal saturation in test tanks;
SS = supersaturation in acclimation and test tanks.

TABLE 4.—Mean values[a] of lethal exposure time (in minutes) for coho salmon that had been acclimated to 15° C water temperature and then subjected to various increases in water temperature and saturation of nitrogen gas.

Dead fish in sample	Level of gas[b]	Lethal exposure time at various temperatures (° C)					
		15	20	23	26	27	28
One fish	NN	--	--	--	--	26.5	8.5
	NS	420	265	2,325	222.5	38.5	7
	SN	--	--	--	255	23	10
	SS	70	36	45	29.5	17.5	12.5
Three fish	NN	--	--	--	--	50	10.5
	NS	430	295	297	245	51.5	14
	SN	--	--	--	705	30	14
	SS	175.5	78.5	62.5	38.5	32.5	18
Half of sample	NN	--	--	--	--	62	13.5
	NS	555	502.5	517.5	645	79	20.5
	SN	--	--	--	855	70	24.5
	SS	247	115	124.5	86	52.5	38
All of sample	NN	--	--	--	--	93	37
	NS	--	--	--	--	115	46
	SN	460	520	930	910	115	48
	SS	519	450	465	312.5	96	38

[a] Mean of replicated tests.
[b] NN = normal saturation (100%) of nitrogen gas in acclimation and test tanks;
NS = normal saturation in acclimation tank and supersaturation (125-130%) in test tanks;
SN = supersaturation in acclimation tank and normal saturation in test tanks;
SS = supersaturation in acclimation and test tanks.

TABLE 5.—Mean values[a] of lethal exposure time (in minutes) for coho salmon that had been acclimated to 20° C water temperature and then subjected to various increases in water temperature and saturation of nitrogen gas.

Dead fish in sample	Level of gas[b]	Lethal exposure time at various temperatures (° C)					
		20	23	25	27	28	29
One fish	NN	--	--	--	93	35	14
	NS	375	558	400	130	29	11
	SN	130	--	15	47	17	7.5
	SS	89	20	135	45	13	9.5
Three fish	NN	--	--	--	104	47	14
	NS	497	510	510	152	38	13
	SN	--	--	--	82	34	13
	SS	180	300	165	69	30	12
Half of sample	NN	--	--	--	121.5	60	20
	NS	810	780	840	197	43	20
	SN	--	--	--	115	53.5	22
	SS	317.5	570	266	115	42.5	16
All of sample	NN	--	--	--	--	93.5	37
	NS	920	1,040	930	910	115	48
	SN	--	--	--	--	115	46
	SS	619	450	465	307	97	38.5

[a] Meon of replicated tests.
[b] NN = normal saturation (100%) of nitrogen gas in acclimation and test tanks;
NS = normal saturation in acclimation tank and supersaturation (125-130%) in test tanks;
SN = supersaturation in acclimation tank and normal saturation in test tanks;
SS = supersaturation in acclimation and test tanks.

TABLE 6.—Mean values[a] of lethal exposure time (in minutes) for hatchery spring chinook salmon that had been acclimated to 15° C water temperature and then subjected to various increases in water temperature and saturation of nitrogen gas.

Dead fish in sample	Level of gas[b]	Lethal exposure time at various temperatures (° C)					
		15	20	23	25	27	28
One fish	NN	--	--	--	90.5	22	5
	NS	510	585	375	210	16.5	4.5
	SN	450	--	390	30	7.5	4
	SS	32.5	30	30	20.5	6.5	3
Three fish	NN	--	--	--	255	55	9
	NS	510	735	510	198	45	8.5
	SN	--	--	--	245	9	6.5
	SS	217	190	110	80	11	4
Half of sample	NN	--	--	--	360	66	115
	NS	675	820	540	255	52.5	10.5
	SN	--	--	--	370	39.5	9
	SS	315	165	123	122.5	16.5	6
All of sample	NN	--	--	--	1,020	88.5	30.5
	NS	900	1,080	755	275	97	25
	SN	--	--	--	700	78.5	19.5
	SS	720	780	525	399	50	40.5

[a] Meon of replicated tests.
[b] NN = normal saturation (100%) of nitrogen gas in acclimation and test tanks;
NS = normal saturation in acclimation tank and supersaturation (125-130%) in test tanks;
SN = supersaturation in acclimation tank and normal saturation in test tanks;
SS = supersaturation in acclimation and test tanks.

TABLE 7.—Mean values[a] of lethal exposure time (in minutes) for hatchery spring chinook salmon that had been acclimated to 20° C water temperature and then subjected to various increases in water temperature and saturation of nitrogen gas.

Dead fish in sample	Level of gas[b]	Lethal exposure time at various temperatures (° C)					
		20	23	25	27	28	29
One fish	NN	--	--	720	34	33.5	5
	NS	255	150	--	71.5	38.5	9.5
	SN	--	--	41.5	6	8.5	5.5
	SS	30	30	7.5	15	10.5	3
Three fish	NN	--	--	--	84.5	44.5	6.5
	NS	360	345	372	86	43.5	14.5
	SN	--	--	795	39.5	16	7.5
	SS	45	67.5	30	28	16.5	4.5
Half of sample	NN	--	--	--	95	51.5	8
	NS	450	435	570	97	48.5	17
	SN	--	--	620	66	23	8.5
	SS	75	176	42.5	44	22	6.5
All of sample	NN	--	--	--	153.5	63.5	13.5
	NS	780	690	750	133	66	27.5
	SN	--	--	820	110	46.5	19
	SS	397	675	600	97.5	52.5	13.5

[a] Meon of replicated tests.
[b] NN = normal saturation (100%) of nitrogen gas in acclimation and test tanks;
NS = normal saturation in acclimation tank and supersaturation (125-130%) in test tanks;
SN = supersaturation in acclimation tank and normal saturation in test tanks;
SS = supersaturation in acclimation and test tanks.

TABLE 8.—Mean values[a] of lethal exposure time (in minutes) for wild spring chinook salmon that had been acclimated to 10° C water temperature and then subjected to various increases in water temperature and saturation of nitrogen gas.

Dead fish in sample	Level of gas[b]	Lethal exposure time at various temperatures (° C)					
		10	15	20	23	25	27
One fish	NN	--	--	61	118	9	4
	NS	600	645	463	163	17	3
	SN	--	98	312	4	2	1
	SS	38	35	68	10	3	1
Three fish	NN	--	--	--	--	192.5	27
	NS	--	--	--	720	465	43
	SN	--	--	--	--	80	9
	SS	660	660	600	780	71.5	6
Half of sample	NN	--	--	--	--	82.5	5
	NS	--	--	780	405	195	4
	SN	--	--	--	1,080	7.5	2
	SS	495	187	150	58	7.5	3
All of sample	NN	--	--	--	--	193	27
	NS	--	--	--	900	165	43
	SN	--	--	--	--	80	9
	SS	870	660	600	780	71	6

[a] Mean of replicated tests.
[b] NN = normal saturation (100%) of nitrogen gas in acclimation and test tanks;
NS = normal saturation in acclimation tank and supersaturation (125-130%) in test tanks;
SN = supersaturation in acclimation tank and normal saturation in test tanks;
SS = supersaturation in acclimation and test tanks.

TABLE 9.—Mean values[a] of lethal exposure time (in minutes) for steelhead trout that had been acclimated to 10° C water temperature and then subjected to various increases in water temperature and saturation of nitrogen gas.

Dead fish in sample	Level of gas[b]	Lethal exposure time at various temperatures (° C)					
		10	15	20	23	25	27
One fish	NN	--	--	930	458	60	4.5
	NS	195	172.5	230	230	60	8.5
	SN	--	360	--	10	6	6
	SS	57.5	15	6	6	8.5	5.5
Three fish	NN	--	--	--	--	200	10
	NS	280	217	367	320	112	15
	SN	--	--	--	--	206	9
	SS	76	50	71	52	19	11
Half of sample	NN	--	--	--	--	225	14
	NS	307	340	493	450	165	17
	SN	--	--	--	--	366	14
	SS	104	75	105	74	38	15
All of sample	NN	--	--	--	--	675	34
	NS	660	660	780	690	465	80
	SN	--	--	--	--	--	52.5
	SS	347.5	307.5	202.5	202.5	95	30

a Mean of replicated tests.
b NN = normal saturation (100%) of nitrogen gas in acclimation and test tanks;
NS = normal saturation in acclimation tank and supersaturation (125-130%) in test tanks;
SN = supersaturation in acclimation tank and normal saturation in test tanks;
SS = supersaturation in acclimation and test tanks.

TABLE 10.—Mean values[a] of lethal exposure time (in minutes) for steelhead trout that had been acclimated to 15° C water temperature and then subjected to various increases in water temperature and saturation of nitrogen gas.

Dead fish in sample	Level of gas[b]	Lethal exposure time at various temperatures (° C)					
		15	20	23	26	27	28
One fish	NN	--	--	--	225	23	18
	NS	570	480	480	207	37.5	15
	SN	--	--	--	7	9.5	6
	SS	15	25	14	14	8	4.5
Three fish	NN	--	--	--	660	62.5	21
	NS	645	570	600	390	53.5	20
	SN	--	--	--	151.5	16.5	11.5
	SS	30	56	30	21.5	14	11
Half of sample	NN	--	--	--	--	75	24
	NS	840	630	645	435	58	23.5
	SN	--	--	--	495	23.5	15
	SS	45	94	52.5	43	19	19
All of sample	NN	--	--	--	--	107.5	42
	NS	1,320	870	900	720	81.5	34.5
	SN	--	--	--	840	127.5	32.5
	SS	690	452.5	157.5	225	57.2	24.5

a Mean of replicated tests.
b NN = normal saturation (100%) of nitrogen gas in acclimation and test tanks;
NS = normal saturation in acclimation tank and supersaturation (125-130%) in test tanks;
SN = supersaturation in acclimation tank and normal saturation in test tanks;
SS = supersaturation in acclimation and test tanks.

stressed fish were subjected to temperature increases in water supersaturated at 125 to 130%, however, the prior stress significantly decreased their tolerance at temperatures below 26° C. Figure 1 shows only the data from populations acclimated to 15° C (Tables 3, 6, and 10), but similar curves that clearly show the effect of supersaturation can be constructed from the data on populations acclimated to the other temperatures.

Populations of coho salmon, steelhead trout, and chinook salmon acclimated to higher temperatures were able to tolerate higher temperatures for longer periods in supersaturated water as well as in normally saturated water. A comparison of our LE_{50} curves for coho with those of Brett (1952) indicates that tolerance to higher temperatures in supersaturated water

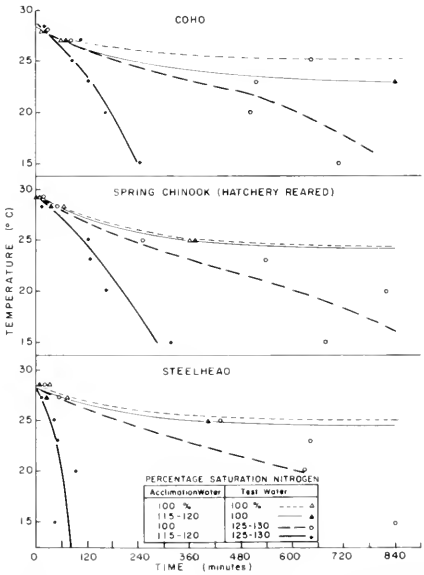

FIGURE 1.—Comparison of LE_{50} curves of hatchery-reared juvenile coho salmon, spring chinook salmon, and steelhead trout acclimated at 15° C and stressed at various levels of nitrogen saturation.

was considerably lower at exposures over 100 min for the same acclimation temperature than at normal saturation levels (Figure 2).

A comparison of the NN and SN curves (Figure 1) indicates that the prior stress of supersaturation of nitrogen had little effect on the fish when they were subjected to test water that was not supersaturated. This suggests that migrating salmon and trout under stress from supersaturation of nitrogen gas could recover from the effects of supersaturation if there were river areas where water would equilibrate. These data also show that salmon and trout populations acclimated at 15° C and subjected to nitrogen saturation of 125 to 130% will probably have about 50% mortality in less than 360 min with no temperature increase when stressed for 12 hr before testing and that subjecting the populations to temperature increases merely reduces the time to death.

In comparing the tolerance to temperature increases between coho and spring chinook salmon, we found that the results from the control tests—where both acclimation and test water were at 100% saturation—were similar to the results of Brett (1952). That is, coho were more tolerant than chinook and the respective upper lethal temperatures were 25 to 26° C. Brett did not study steelhead trout. We found that steelhead trout were nearly identical to coho in their tolerance to temperature increases when supersaturation of nitrogen was not present (control tests) but were the most vulnerable species when supersaturation was entered as a factor. Figure 3 compares the tolerance of three species to temperature increases when acclimation water and test water were supersaturated. Coho were the most tolerant, wild spring chinook next, and steelhead the least tolerant.

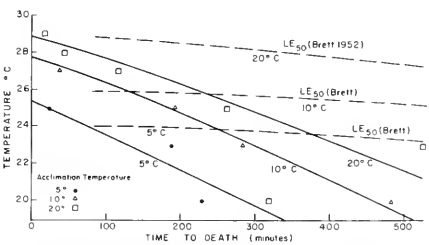

FIGURE 2.—Median-resistance-time (LE_{50}) plotted from temperature tolerance tests with coho salmon fingerlings acclimated at 5°, 10°, and 20° C and stressed for 12 hr at 115-120% saturation of N_2, then subjected to temperature increases in water supersaturated at 125-130%. Brett's LE_{50} curves for fish acclimated at 5° and 20° C—without N_2 stress—are shown for comparison.

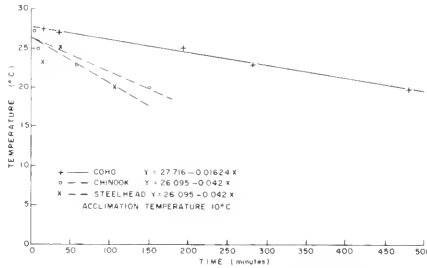

FIGURE 3.—Comparison of LE_{50} values between species of salmonid juveniles (acclimated at 10° C) in tolerance to temperature increases when stressed by 115-120% saturation of nitrogen gas for 12 hr, then subjected to temperature increases in supersaturated water at 125-130% nitrogen.

Size of fish at time of testing could influence the comparison between species. Coho and wild spring chinook acclimated at 10° C were nearly the same size at time of testing (Table 1), but steelhead acclimated at 10° C were larger than the other species when tested. There is evidence that extremely small chinook fry are more susceptible to nitrogen supersaturation than fingerlings; then, as the fingerlings increase in size, they become more susceptible than the small fingerlings but less susceptible than the fry (Meekin, 1969).[2] If this occurs with steelhead also, it could account for their lower tolerance. During one test with coho acclimated at 15° C, we found no differences in susceptibility within the size range tested (101-151 mm) at 125 to 130% saturation.

[2] Personal communication, Thomas Meekin, Washington State Department of Fisheries. Experiments at Priest Rapids Dam.

We intended to compare hatchery-reared chinook salmon with wild or naturally migrating spring chinook salmon acclimated to the same temperature. Wild fish were available only when they were acclimated to temperatures near 10° C. The hatchery population had deteriorated by then, however, so we compared wild fish acclimated at 10° C and hatchery fish acclimated to 15° C (Figure 4). As expected, the hatchery population—acclimated at the higher temperature—was able to tolerate the highest temperatures for a longer period, but when the LE_{50} curves—which include the effect of nitrogen—are compared, little difference can be noted. This indicates that results achieved in the laboratory with hatchery stocks can be applied to wild Columbia River stocks with reasonable accuracy.

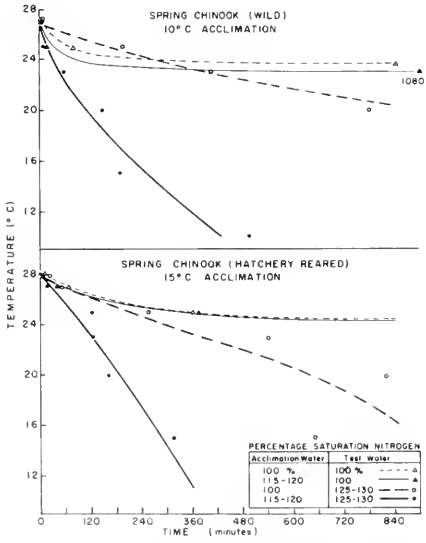

FIGURE 4.—Comparison of LE_{50} curves between juvenile wild and hatchery spring chinook salmon at various temperatures and levels of saturation of nitrogen gas.

EFFECT OF DEPTH ON RELATION BETWEEN SUPERSATURATION OF NITROGEN AND TOLERANCE OF JUVENILE FISH TO TEMPERATURE INCREASES

Examination of fish in cages at the forebay of Priest Rapids Dam (Ebel, 1969) indicated that juvenile coho and chinook salmon would not contract gas bubble disease if held at a sufficient compensating depth (5 m). This finding suggests that fish subjected to temperature increases in addition to nitrogen supersaturation would also be less affected if they remained at sufficient depth when they encountered a temperature increase.

To test this hypothesis, we subjected coho salmon acclimated at 10° C to three temperatures above acclimation in water supersaturated at 130% in the 9-m (deep) tank where they could select any depth from the surface to 9 m; we then compared LE_{100} curves in the 20-cm (shallow) tanks with those in the deep tank (Figure 5). These curves definitely indicate that the coho benefited by having the option to sound in the deep tank. The LE_{100} level never was reached during the 18-hr observation period when the fish were subjected to 20° C (10° C increase) in the deep tank, but occurred after

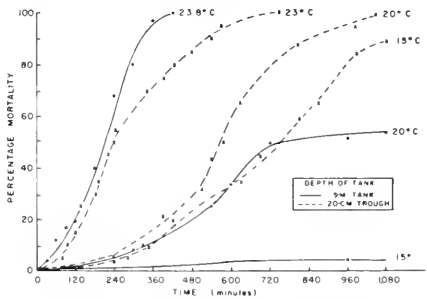

FIGURE 5.—Comparison of LE_{100} curves for coho salmon acclimated at 10° C and subjected to three temperatures (15°, 20°, and 23° C) in 20-cm and 9-m deep tanks containing water supersaturated with nitrogen gas at 130% saturation. Oxygen concentrations varied from 115 to 125% saturation.

about 17 hr in the shallow tank. Similarly at 15° C (5° C increase), the LE_{50} level was never reached in the deep tank but was reached in about 12.5 hr in the shallow tank. No benefit from depth is indicated in the curves at 23° C; in this comparison, the fact that temperature in the deep tank was 0.8° C higher (23.8° C) than that in the shallow tank could account for the lack of difference.

Wild juvenile spring chinook salmon from the gatewells at McNary Dam also were tested in the deep and shallow tanks. These fish were acclimated at 10° C and then subjected to a 5° C increase (15° C) with supersaturation of nitrogen gas at 130% saturation. The fish also were stressed for 12 hr before the test in 10° C water supersaturated at 120% saturation. Again, chinook tested in the deep tank survived at a higher rate than those in the shallow tanks; the LE_{50} was never reached in the deep tank, whereas 100% mortality was reached in approximately 11 hr in the shallow tanks (Figure 6).

Observations in the deep tank during tests with the coho and chinook salmon indicated that most fish remained between about 1 and 4 m of the surface. Light intensity and turbidity possibly influenced the depth distribution. During these tests, artificial light at an intensity of about 100 footcandles was present at the surface of the water. Turbidity in the tank was minimal; a Secchi disc was visible at the bottom of the tank and the Jackson turbidity unit measurement was 0.

It is difficult to relate tests in the tank to natural conditions because turbidity in natural water varies greatly. In the Snake River, turbidity as measured by a Secchi disc varies from 0.2 to 8.0 m, depending on season and location. Turbidity usually is high during the spring runoff in both the Snake and Columbia Rivers; readings are seldom over 1 m on the Snake River (Ebel and Koski, 1968). This high turbidity limits visible light penetration to a maximum of about 1.5 m (observation verified by scuba diving). We therefore believe that juveniles as observed in the tank were at greater depths than they might be in the Snake or Columbia Rivers during the spring migration. Durkin, Park, and

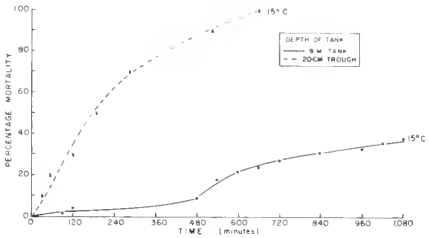

FIGURE 6.—Comparison of LE_{100} curves of wild spring chinook salmon acclimated at 10° C and subjected to a 5° C increase (15° C) in tanks 20 cm and 9 m deep that were supersaturated with nitrogen gas at 130% saturation. Oxygen concentrations varied from 115 to 125% saturation.

Raleigh (1970) found that most juvenile salmon were near the surface as they entered Brownlee Reservoir. Fish in the Columbia and Snake Rivers apparently do not sound to a depth sufficient to compensate for nitrogen saturation levels exceeding 130%; hence the mortalities reported herein are probably on the conservative side. We also emphasize that even though the option of having sufficient depth reduced the mortality rate, substantial mortalities occurred.

TEMPERATURE STANDARDS FOR RIVERS WITH NITROGEN SUPERSATURATION

Our test temperatures and experimental design were purposely selected so that these data could be compared with the results reported by Brett (1952). Brett cautions that the information he presents should not be applied verbatim to other environments. Because of the excellence of his work and the lack of later findings concerning temperature tolerance of Pacific salmon, the upper lethal levels established in his paper are widely quoted and used for setting temperature tolerance standards for rivers and streams containing salmon—without regard to other physical and chemical characteristics of the water. The changes in Brett's tolerance curves caused by the stresses of supersaturation of nitrogen gas were obvious.

Although complete statistical analysis of our data are not presented in this paper, the differences shown between tolerance curves of fish tested in water with and without supersaturation of nitrogen are so great that conclusions concerning the effect of supersaturation can be made with relative confidence.

Substantial mortalities will occur to migrating juvenile salmon and trout in the Columbia and Snake Rivers—even if no thermal plume or increase in temperature is encountered—whenever the populations must pass through large areas where 125 to 130% saturation of nitrogen occurs. Studies of vertical distribution (e.g., Smith, Pugh, and Monan, 1968; Durkin et al., 1970) indicate that the majority of migrants are in surface waters, with substantial numbers in waters less than 2 m deep. This is too shallow to compensate for nitrogen levels as high as 130%. Surveys of nitrogen levels by Ebel (1969), by Beiningen and Ebel (1971), and by NMFS and State fisheries personnel of Washington and Oregon during the 1970 spring migration, verify that nitrogen in large areas of both rivers exceed 130% saturation. Examination of fish in cages suspended on the surface and at various depths revealed that mortalities caused by nitrogen often exceeded 40% in a deep (4.5 m) cage where the fish could sound at their volition. Periodic checks of juveniles in the Snake River by NMFS personnel in 1970 indicated that 25 to 45% of the chinook salmon and 30 to 58% of the steelhead trout migrants arriving at Ice Harbor Dam had external symptoms of gas bubble disease. We made similar observations of migrants at The Dalles and McNary Dams in 1968 and 1969 and recorded similar findings.

Obviously the migrating juvenile salmon and trout in the Columbia and Snake Rivers are under stress during periods of nitrogen supersaturation. Any increase in temperature over the ambient river temperature, then, will harm these populations. Mortalities already occurring will be accelerated even with minimal temperature increases. Our data show that LE_{50} levels of temperature (Figures 1-4) are far higher than could be accepted as standards for upper limits of rivers containing trout and salmon even at normal concentrations of dissolved nitrogen. The time to first mortality of wild spring chinook salmon, for example, that were acclimated to 10° C and tested in supersaturated water at 23° and 25° C was 10 and 3 min, respectively (Table 8). Temperatures and temperature increases such as these occur in thermal effluents (Coutant, 1969), and substantial mortalities could occur to juvenile salmon and trout passing through thermal plumes.

During spring and summer when flows are low, increases in temperature of the Columbia River from Priest Rapids Dam to the forebay of McNary Dam have been as high as 2.5° C (Ebel, 1969). Increases in temperature over the acclimated temperature greatly accelerated time to death of juveniles when supersaturation of nitrogen gas was present in the test water whether the fish were held in shallow or deep tanks. However, during the low flow periods when temperature increases such as this occur, nitrogen saturation levels are usually low and mortalities such as indicated in the tests would not occur.

The obvious results of these tests are that supersaturation of nitrogen must be considered when setting temperature standards and that any increase allowed over the ambient temperature of the river during periods when the river is supersaturated with nitrogen will be detrimental to salmon and trout populations.

CONCLUSIONS

1. Supersaturation of nitrogen drastically affects the tolerance of juvenile coho salmon, chinook salmon, and steelhead trout to temperature increases. Tolerance to increases below 26° C is lowered and mortality rates are accelerated.

2. Acclimation to higher temperatures will enable the three species to tolerate higher temperatures longer when nitrogen supersaturation is a factor; however, 50% mortality will be reached in less than 18 hr at all acclimation temperatures with supersaturation of nitrogen at 125 to 130%. No temperature is suitable at the 125 to 130% level of nitrogen supersaturation.

3. Depth is an important compensating factor

when supersaturation of nitrogen is present. Tests in the deep (9-m) tank, where fish were free to roam from the surface to the bottom, revealed that mortality rates were much lower and tolerance to temperature increases was increased if the juveniles had the option to sound when subjected to temperature increases.

4. Coho were the most tolerant, chinook next, and steelhead the least tolerant to temperature increases when the water was supersaturated with nitrogen. When supersaturation was not a factor, coho and steelhead were about equally tolerant to temperature increases and chinook the least tolerant.

5. Any increase in temperature allowed over the ambient temperature (whether high or low) of the river during periods of supersaturation of nitrogen will be detrimental to migrating juvenile salmon and trout. Temperature standards should account for the effect of supersaturation of nitrogen gas.

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CONTRIBUTION TO THE POPULATION DYNAMICS OF ATLANTIC ALBACORE WITH COMMENTS ON POTENTIAL YIELDS[1]

GRANT L. BEARDSLEY[2]

ABSTRACT

Length-frequency data on Atlantic albacore from the Bay of Biscay surface fishery and the Atlantic longline fishery were analyzed. Lengths at age were estimated and the von Bertalanffy growth parameters were calculated: $K = 0.141$, $L_\infty = 140$ cm, and $t_0 = -1.63$ years. Instantaneous rates were computed on an annual basis. The total instantaneous mortality coefficient was estimated as 0.96 for albacore in the Bay of Biscay fishery and 0.79 in the longline fishery. Analysis of catch and effort data suggested that greater yields are available from the North and South Atlantic longline stocks though stock identification in the South Atlantic is not clear. Estimates of population structure in the North Atlantic were made by utilizing total instantaneous mortality rates of 0.50, 0.96, and 1.40 and an instantaneous natural mortality rate of 0.23. The population based on a total mortality coefficient of 0.96 appeared to be the most reasonable.

The albacore, *Thunnus alalunga*, has become increasingly important to the Atlantic tuna fisheries in recent years. From 1956 to 1961 the Japanese longline fishery in the Atlantic was primarily directed at yellowfin tuna, *T. albacares*, but rapidly declining catch rates for yellowfin soon forced a shift of fishing into primarily albacore areas (Wise, 1968). As a result of decreased yellowfin catches and a corresponding shift in fishing toward albacore, the average number of albacore caught yearly by the Japanese in the Atlantic increased from 228,000 in the years 1956-61 to 1,332,857 in the years 1962-68. The percentage of albacore in the combined catch of albacore and yellowfin in the Atlantic by the Japanese rose from an average of about 18.7% in 1956-61 to 67.7% in 1962-68 (Figure 1).

Since 1965 the Japanese have significantly curtailed their longline fishing in the Atlantic. From a high of almost 100 million hooks in 1965, they set slightly over 30 million hooks in 1967 and again in 1968. This decrease, however, has been offset by the entry of China (Taiwan) and South Korea into the fishery as well as small amounts

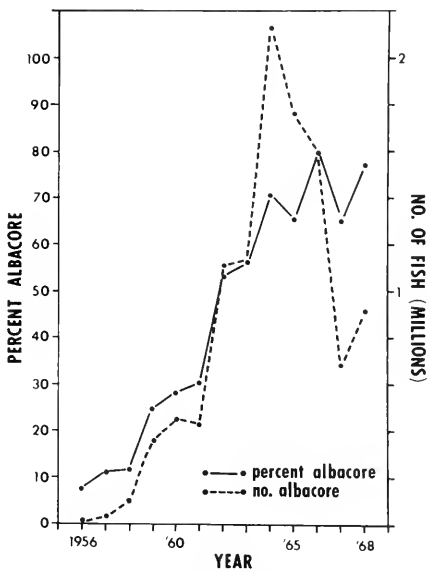

FIGURE 1.—Total number of albacore caught and the percentage of albacore in the combined yearly albacore-yellowfin tuna catch by Japanese longliners in the Atlantic Ocean, 1956 through 1968.

[1] Contribution No. 203, National Marine Fisheries Service, Southeast Fishery Center, Miami, Fla. 33149.

[2] National Marine Fisheries Service, Southeast Fishery Center, Miami, Fla. 33149.

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of fishing by Cuba and Venezuela. In 1968 the combined landings of albacore by China and South Korea were about 15,500 metric tons, which was only 1000 tons less than the Japanese catch for 1968. Recent estimates have placed the Atlantic albacore catch for 1969 by China and South Korea at 19,300 metric tons.

Worldwide demand for tuna is increasing every year, and there is growing concern over the condition and well being of Atlantic tuna stocks. Catch rates of yellowfin tuna in the longline fishery suffered severe declines in the 1960's. Wise (1968), Food and Agriculture Organization (1968), Wise and Fox (1969), Hayasi and Kikawa (1970), and others have examined the problem and have concluded that the longline stocks of yellowfin tuna have been fished beyond their maximum capacity.

This paper is an analysis of some aspects of the population dynamics of Atlantic albacore with comments on potential yields and stock size.

AGE AND GROWTH

There have been few studies on the age and growth of Atlantic albacore. Distinct modes appear in length-frequency distributions of albacore samples from the Bay of Biscay surface fishery, and these modes probably represent age-groups. There is considerable disagreement, however, over the assignment of absolute ages to Atlantic albacore (Table 1).

Le Gall (1949, 1952) worked with length frequencies from the Bay of Biscay. He stated that age-group I was less than 48 cm in length, and that the first group that appeared in the fishery was age-group II (56 cm). Figueras (1957) used vertebrae from 67 fish in his analysis and concluded that 56- to 57-cm albacore were 4 years old and that 17- to 18-cm albacore were 1 year old. Yang (1970) estimated that 56-cm albacore were approximately 3 years old based on results of his analysis of annular markings on scales. Otsu and Uchida (1959b), however, concluded from their study of vertebrae, scales, and other hard parts of Pacific albacore that there were no markings that could be considered age marks.

I used data from the Bay of Biscay for 1967, 1968, 1969, and 1970 (Allain and Aloncle, 1968; Philippe Serene and Jean-Claude Dao, Centre National pour l'Exploitation des Océans, personal communication) (Figure 2) as well as longline data (Figure 3) and estimated lengths at age for Atlantic albacore (Table 1).

Lengths at ages 1, 2, 3, and 4 were estimated by modes in length-frequency histograms from Bay of Biscay samples. The first mode distinguishable in the length frequencies (Figure 2, 1968) is at approximately 44 cm, and I assume that it represents age-group I. There is support in the literature for the assignment of this approximate length to 1-year-old albacore. Otsu and Uchida (1963) indicated that 30- to 35-cm

TABLE 1.—Summary of age and growth investigations on Atlantic albacore.

Investigator	Method	Sample size	Age (years)									
			1	2	3	4	5	6	7	8	9	10
			cm									
Prial (1944)	Scales (total length)	50	50-58	59-74	74-86	86-94	94-98	--	--	--	--	--
Le Gall (1949)	Length frequencies (total length)	--	<25	25-46	46-60	60-74	74-88	--	--	--	--	--
Le Gall (1952)	Length frequencies (total length)	50,000	<48	56	68	81-82	>93	--	--	--	--	--
Figueras (1957)	Vertebrae (total length)	67	17-18	31-32	44-45	56-57	69-70	81-82	91-93	--	--	--
Yang (1970)	Scales (fork length)	159	20.3	39.6	56.1	71.2	80.9	90.3	98.1	--	--	--
Beardsley (present study)	Length frequencies (fork length)	62,602	44	55	64	75	87	95	100	104	108	112

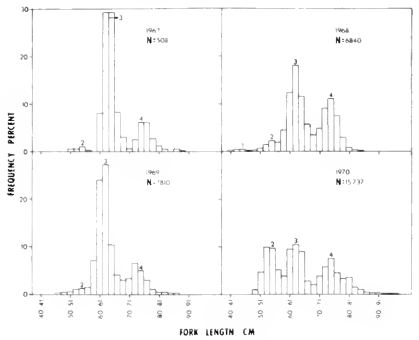

FIGURE 2.—Albacore length-frequency distributions from the Bay of Biscay surface fishery, 1967-70. Estimated ages in years are shown above the histograms.

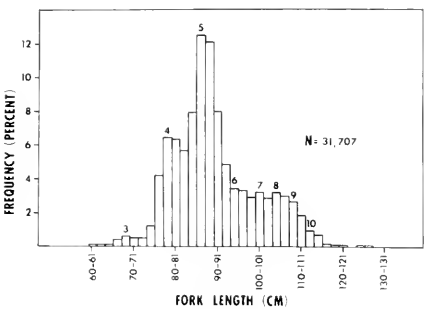

FIGURE 3.—Albacore length-frequency distribution from the Atlantic longline fishery, several years combined. Estimated ages in years are shown above the histograms.

albacore occasionally appear in the Japanese live-bait fishery in spring, and they assume that these fish are approximately 1 year old. On this basis they assign 2, 3, and 4 years of age to 55-, 65-, and 76-cm albacore respectively in the United States west coast fishery. Yoshida (1968) studied age and growth of juvenile albacore based on 35 specimens recovered from stomachs of billfishes in the Pacific. He derived a hatching date of May 1 based on a regression analysis of length of the juveniles against month of capture and concluded that 1-year-old albacore are approximately 38 cm standard length. Although I have assigned a length of 44 cm to age-group I, a more practical approach would probably be to accept Le Gall's (1952) statement that 1-year-old albacore are "less than 48 cm in length."

Longline length frequencies were used to estimate ages 5 and older (Figure 3). The assignment of ages beyond age 5 is somewhat subjective. I assigned ages 7 and 8 to the modes appearing at 100 to 101 and 104 to 105 cm. Other ages were assigned mostly by extrapolation. My estimates agree in most cases with those made for Pacific albacore by Otsu and Uchida (1963). No attempt was made to assign ages beyond age 10 although undoubtedly some albacore in the Atlantic live to be older than 10.

The two modes which correspond to ages 3 and 4 in the longline samples (68-69 and 78-79 cm) are located at a length of 3 to 6 cm greater than the same age in the Bay of Biscay samples. Yang (1970) stated that ring formation on albacore scales occurred in February-March for North Atlantic albacore. The Bay of Biscay samples were taken in summer, and the albacore had completed approximately a half year's growth. Most 3- and 4-year-old albacore captured by longliners are taken in winter when they are first recruited to the fishery. These 3- and 4-year-old fish are at the end of a year's growth or just beginning a new year's growth, and the disparity in the position of the modes between the longline samples and the Bay of Biscay samples represents growth during the period between the summer fishery and the winter fishery.

GROWTH PARAMETERS

I constructed a Walford line (Figure 4) using the lengths at age from this study (Table 1) and took the intercept of the 45° diagonal as an initial trial value for L_∞ in the expression for growth (von Bertalanffy, 1938):

$$\log_e (L_\infty - L_t) = \log_e L_\infty + Kt_0 - Kt.$$

A best fit was obtained with $L_\infty = 140$ cm. K was calculated as 0.141, and t_0 was −1.63 years. Yang (1970) found $L_\infty = 135$ cm and K = 0.19

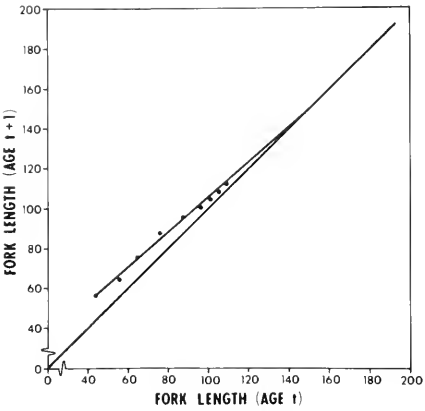

FIGURE 4.—Walford transformation of length in centimeters at age $t + 1$ against length at age t for Atlantic albacore.

from his analysis of albacore growth. The growth curve based on my calculations is shown in Figure 5.

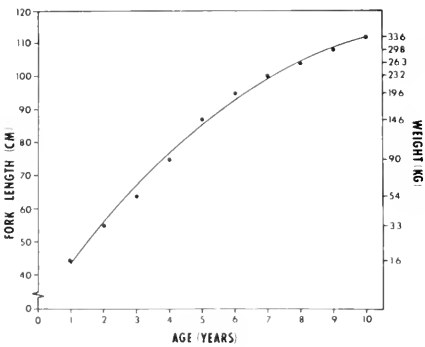

FIGURE 5.—Growth curve for Atlantic albacore.

LENGTH-WEIGHT

De Jaeger (1963), de Jager, Neppgen, and van Wyk (1963), van den Berg and Matthews (1969), and Nepgen (1970) published information on the length-weight relation of Atlantic albacore. All gave separate equations for males and females although de Jaeger (1963) was unable to detect any significant differences in his samples. I combined data from the long-line fishery and the Bay of Biscay fishery and calculated a length-weight equation for both sexes combined since sex information was not available for most of the samples at the smaller sizes:

$$W = 6.303 \times 10^{-6} \times L^{3.28253}.$$

Weight is in kilograms and length is fork length in centimeters (Figure 6).

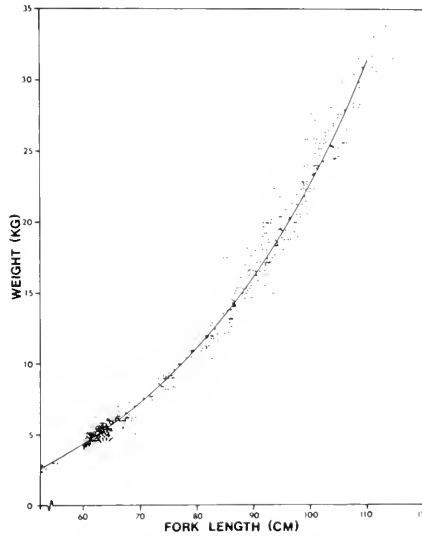

FIGURE 6.—Length-weight relation of Atlantic albacore, both sexes combined.

SEX RATIO

Five hundred ninety-eight albacore were measured and sexed at canneries located in Puerto Rico from December 1969 to September

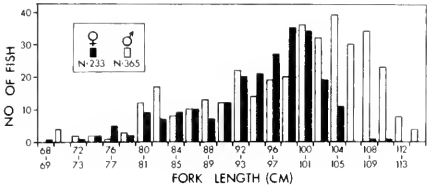

FIGURE 7.—Length-frequency distribution of 598 male and female albacore measured at canneries in Puerto Rico from December 1969 to September 1970. All were caught in the Atlantic by longline gear.

1970. Males constituted 61% of the samples and were more abundant at the larger sizes (Figure 7). Few females appear to attain a length of much over 100 cm. This dominance of males both in numbers and at the larger sizes has been reported for Pacific albacore by Otsu and Uchida (1959a, 1959b) and Otsu and Hansen (1962) and for Atlantic albacore by de Jaeger (1963) and Talbot and Penrith (1963).

MORTALITY

There have been no mortality estimates for Atlantic albacore that I am aware of. Suda (1963) stated that the total instantaneous mortality coefficient of North Pacific albacore is probably around 0.4. He later estimated that the natural mortality coefficient for the same stock is about 0.2 (Suda, 1966). I estimated the total instantaneous mortality coefficient (Z) for Atlantic albacore using Bay of Biscay length frequencies (Figure 2) and longline length frequencies (Figure 3). All mortality rates in the following discussion are instantaneous rates unless specifically designated as annual.

BAY OF BISCAY

If we assume that the 1967, 1968, 1969, and 1970 samples from the Bay of Biscay are reasonably representative of the total catch which is in turn an accurate estimator of the true relative abundance of the different age groups in the fishery, we can calculate a total mortality coefficient from the decline in abundance of a given year class from one year to the next, beginning with the first year it is fully recruited (age 3). This method requires weighting the frequencies by catch per unit of effort (CPUE). Accurate estimates of CPUE for the entire Bay of Biscay fishery are not available, although recent research suggests that a slight decline in CPUE has occurred at selected ports in France in the late 1960's (Jean-Claude Dao, personal communication). I have assumed a constant CPUE over the 4 years in question for the purpose of this analysis since complete figures are not available.

I have also assumed equal recruitment for ages 3 and 4 though this is not likely. Any mortality estimates based on the relative abundance of ages 3 and 4 in the fishery will be affected by relative differences in recruitment. Some 3-year-old albacore and many 4-year-olds are recruited to the winter longline fishery in the North Atlantic; however, for the 4 years, 1965-68, the average number of 4-year-old albacore caught in the North Atlantic winter fishery was only about 40,000. This is a relatively insignificant number when compared with the total number of 4-year-olds available in the Bay of Biscay fishery. It is not known if all the survivors return to the Bay of Biscay the following summer although it is probable that most of them do. If 4-year-old albacore do not all return to the Bay of Biscay from the winter longline grounds then mortalities will be slightly overestimated.

Mortality coefficients were calculated from the decline in abundance from age 3 to age 4 only, and the frequency polygons were divided in the following manner. Where there was an obvious null between age classes, for example at 70 to 71 cm in the 1967 plot, the number of fish in that length group were evenly divided, half were assigned to the age above and half to the age below. Where there was no obvious null, as between ages 2 and 3 in the 1969 plot and between ages 4 and 5 in almost all the plots, the dividing point was placed at a length approximately half way between the assigned lengths at age obtained from

Table 1. Using this method I obtained the following values of Z:

Year class	Z
1964 (3 years old in 1967)	0.73
1965 (3 years old in 1968)	1.04
1966 (3 years old in 1969)	1.12

The average total mortality coefficient over the 3 years was estimated to be 0.96.

LONGLINE FISHERY

A total mortality coefficient was calculated for albacore in the longline fishery using a formula derived by Beverton and Holt (1956):

$$Z = \frac{K\,(L_\infty - \bar{L})}{\bar{L} - L_r}$$

where $\bar{L}$ is the average length of the fish in the catch that are as large as or larger than the first fully recruited length, L_r.

Using: $L_\infty = 140$ cm
$K = 0.141$
$\bar{L} = 94.2$ cm
$L_r = 86$ cm

then:

$$Z = 0.79$$

The total mortality coefficient is not incompatible with the average Bay of Biscay estimate if we consider that the longline is probably relatively inefficient compared with surface gear; hence fishing mortality in the longline fishery and consequently total mortality (assuming natural mortality stays nearly the same throughout the life span of the fish) is probably less than in the surface fishery.

The albacore samples used to estimate mortality in the longline fishery were taken over several years from different areas in the Atlantic. It is very likely, however, that the composite length-frequency distribution in Figure 3 is not a completely accurate picture of the length composition of albacore in the Atlantic. Size distribution in the winter fishery, for example, is entirely different from that in the summer fishery, and suitable samples from each fishery would have to be taken to ensure a representative picture over the entire ocean.

YIELD ESTIMATES

There are five major areas in the Atlantic where longliners concentrate on albacore. I have designated these areas A through E in Figure 8 and for ease of discussion will subsequently refer to these areas by their letter designation. Areas A, B, C, and D were described by Beardsley (1969) and Koto (1969) as major fishing areas. Area E off the coast of Argentina, Uruguay, and southern Brazil has only recently developed into a relatively major albacore fishing area. Of the four areas discussed by Beardsley and Koto, only area C has shown a decline over the years in catch rate (Figure 9). Areas A and D produce fish that are relatively small for longline fish and presumably are recruits to the longline fishery. Recent size data obtained from albacore landed in Puerto Rico and caught in area E reveal that small albacore are also a large part of the catch in this area.

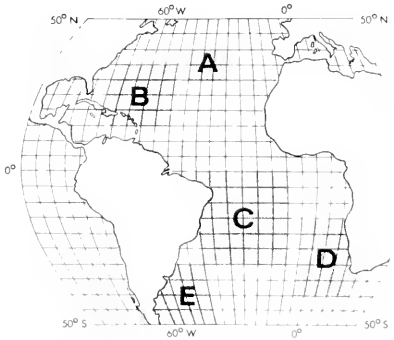

FIGURE 8.—The major longline fishing areas for albacore in the Atlantic Ocean.

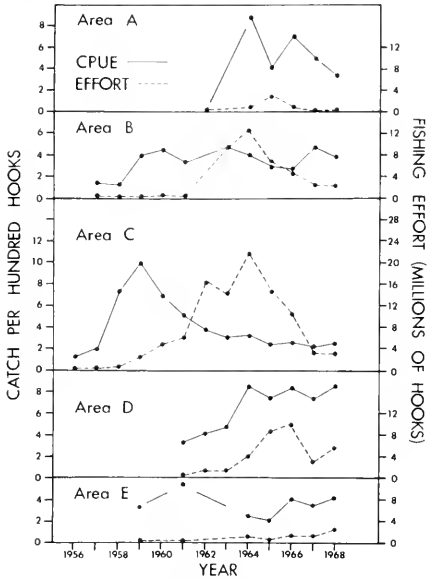

FIGURE 9.—Total longline effort and catch per unit of effort (CPUE) for the five major albacore fishing areas in the Atlantic Ocean, 1956-68.

NORTH ATLANTIC

The North Atlantic supports two different albacore fisheries, the longline fishery and the Bay of Biscay surface fishery, while the South Atlantic has only the longline fishery. The Bay of Biscay fishery, conducted primarily by the French and Spanish, has yielded about 45,000 metric tons annually since 1963. The fishery catches primarily 3- and 4-year-old albacore before they are fully recruited into the longline fishery.

There is evidence that albacore stocks in the North and South Atlantic are separate (Beardsley, 1969; Koto, 1969; Yang, Nose, and Hiyama, 1969). Koto indicates, however, that there may be some mixing of immature albacore between the South Atlantic and Indian Oceans. I combined catch and effort data from the two major albacore fishing areas in the North Atlantic and from the three areas in the South Atlantic and treated each group as separate stocks.

From 1957 through 1965 I used only Japanese data (Shiohama, Myojin, and Sakamoto, 1965; Fisheries Agency of Japan, 1966, 1967a, 1967b) since the longline fishery during those years was almost exclusively Japanese. For 1966, 1967, and 1968 I used Japanese data (Fisheries Agency of Japan, 1968, 1969, 1970) as well as estimated Chinese and Korean catch data (Wise, 1970). Catch per unit of effort (CPUE) in this discussion is the number of albacore caught per hundred hooks fished. CPUE was calculated by summing the number of albacore caught in a given area in a given year, multiplying by 100, and dividing by the number of hooks fished in that area during the year. Only the catch data were available from the Chinese and Korean fishery. Total Chinese and Korean fishing effort for 1966, 1967, and 1968 was obtained by using Chinese and Korean albacore landings and Japanese CPUE and back calculating to the number of hooks fished. This procedure assumes that CPUE for the Chinese and Korean fleets was the same as for the Japanese fleet. This is probably not true; however, the difference is not likely to be large. The Chinese and Korean fishing effort in the Atlantic was not great until 1968 and by then their fishing efficiency was probably comparable to that of the Japanese.

One of the more common mathematical models used to express yield from a stock of fish is the equilibrium-yield model used by Graham (1935), Schaefer (1954, 1957), and others. One of the major advantages of this type of model is that it requires only catch and effort data. Assuming a fishery that has attained equilibrium conditions, a plot of CPUE against effort should show a linear decline which will produce a parabolic curve of yield when plotted against effort. Fox (1970) has argued that the relationship between CPUE and effort is more nearly exponential than linear. Both models, however, predict almost identical yields for the ascending limb of the yield curve. The major difference in the two models occurs after theoretical maximum yields have been exceeded. I chose to limit my analysis to the linear model. The Atlantic longline fishery, however, has never been under equilibrium

conditions. I adjusted for this by using a method suggested by Gulland (1961) whereby effort is an average of the current year's effort (X_i) and the effort from some number of preceding years (X_{i-1}, X_{i-2}, X_{i-3} . . . .), depending on the average number of years a year class is available to the fishery. I used for North Atlantic albacore an average of effort in the current year and the two preceding years:

$$\bar{X} = \frac{X_i + X_{i-1} + X_{i-2}}{3}.$$

The results (Figure 10) show that in the North Atlantic there has been only a slight decline in CPUE over the history of the fishery. When actual catch and effort data are plotted on the predicted yield curve, only in 1964 did yield exceed even 50% of the predicted maximum. It is likely, therefore, that an analysis using an equilibrium-yield model for the North Atlantic albacore longline fishery is not feasible since the population abundance (as represented by CPUE) has apparently not declined sufficiently to effectively describe the dynamics of the stock in relation to fishing. Consequently, maximum sustainable yield from the North Atlantic longline fishery is not estimable at this time. It appears, however, that increased fishing will result in increased yield with no major decline in CPUE.

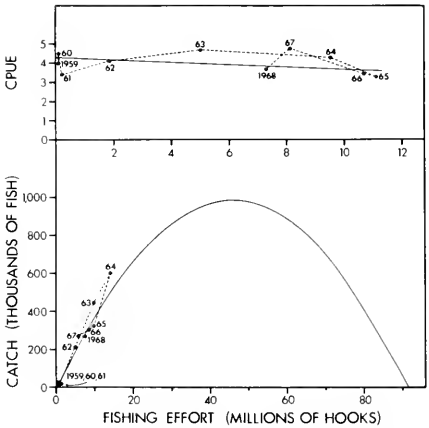

FIGURE 10.—Linear relation between catch per unit of effort (CPUE) and effort (upper panel) and theoretical equilibrium yield curves (lower panel) predicted for the North Atlantic albacore longline fishery. The effort figures in the upper panel are means of the current year's effort and the two preceding year's effort. Effort figures in the lower panel are actual yearly values.

SOUTH ATLANTIC

The albacore longline fishery in the South Atlantic is concentrated in three main areas (Figure 8). During the late 1950's and early 1960's fishing was excellent in area C and fishing effort increased rapidly to a peak of about 22 million hooks in 1964. CPUE declined sharply, however, from a high of 10.0 albacore per hundred hooks, and in recent years has stabilized at about 2.5. In 1961 the Japanese fished in area D for the first time, and this area quickly became the major producer of albacore in the South Atlantic. Fishing is excellent almost year round, and CPUE has remained fairly constant at about 8.0 albacore per hundred hooks over the past 5 years. Area E has recently developed as a good albacore area although effort is still relatively low.

I combined catch and effort data from the Japanese, Chinese, and Korean longline fishery and plotted CPUE against effort in the same manner as for the North Atlantic (Figure 11). The decline in CPUE is more pronounced than for the North Atlantic. The yield curve indicates a theoretical maximum yield of about 1,100,000 albacore from an effort of about 32 million hooks. This yield was equaled in 1964 and surpassed in 1966 and 1968 with an effort of about 25 million hooks.

The South Atlantic albacore fishery has undergone two rather separate and distinct phases. The first phase was from 1956 to 1964 when most fishing effort was in area C. This area produced excellent catches for several years; then catch rates declined rapidly. In 1965 the Japanese increased their fishing in area D in response to excellent catch rates in this area.

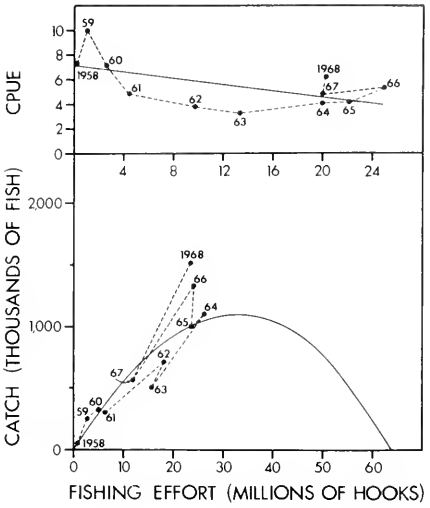

FIGURE 11.—Linear relation between catch per unit of effort (CPUE) and effort (upper panel) and theoretical equilibrium yield curves (lower panel) predicted for the South Atlantic albacore longline fishery. The effort figures in the upper panel are means of the current year's effort and the two preceding year's effort. Effort figures in the lower panel are actual yearly values.

When the data are combined from areas C, D, and E, a decline in CPUE is evident to about 1963 (Figure 11). This decline is primarily in area C. From 1963 to 1968 a steady increase in CPUE is evident which represents the decrease in effort in area C and the increase in effort in area D where excellent catches were being made. It appears from the overall picture of CPUE versus effort that the albacore population in the South Atlantic has declined only slightly in relative abundance as a result of longline fishing.

This may be misleading. I suggested (Beardsley, 1969) that the small albacore in area D formed the recruitment to the South Atlantic population. This hypothesis was based on size differences between the two areas; albacore from area D were small, often averaging as little as 10 to 12 kg, while those caught in area C usually averaged 18 to 20 kg or larger. The monthly distribution of catch rates also indicated seasonal movement between the two areas.

Recent size information obtained from albacore landed in Puerto Rico and caught in area E show that small albacore are a large part of the catch. Recruitment to the South Atlantic population may take place in this area. Koto's suggestion that small albacore move between the Indian and South Atlantic Oceans lends support to this hypothesis. The small albacore in area D may well be transients in the South Atlantic, and any population analysis would have to treat them as a separate stock.

Until the problem of stock identification in the South Atlantic is resolved, any estimates of yield are to be considered tentative. Consistently high CPUE values in area D over the past 5 years suggest that even greater yields are possible from this area. The pronounced decline in CPUE in area C demonstrates a significant response of the population to heavy longline fishing pressure, and any large increase in fishing effort probably would not result in a significant increase in yield.

POPULATION ESTIMATES

I obtained the approximate average number of albacore caught from each age-class in the Bay of Biscay from 1963 through 1968 in the following manner.

Each length-frequency sample from the Bay of Biscay (Figure 2) was separated into age-groups in the same manner as described earlier. The number of albacore in each age-group was then divided by the total number of fish in the respective sample to obtain the percentage contribution of each age-group to the sample. This percentage was considered as being representative of the contribution of that age-group to the total catch for that year. An average percentage contribution over the 4 years, 1967-70, was then calculated for each age-group (Table 2).

I then calculated the total weight of a cohort of 1000 albacore using weights at age (Figure 5) and average percentages obtained above (Table 3). The average annual catch from the Bay of

TABLE 2.—Percentage contribution (in number of fish) of each age-group in the Bay of Biscay from 1967 through 1970.

Age-group	Year				Mean
	1967	1968	1969	1970	
1	00.0	01.1	00.0	00.0	00.3
2	01.9	05.6	03.9	32.5	11.0
3	78.0	54.9	74.6	34.7	60.5
4	16.3	37.5	19.5	24.3	24.4
5	03.8	00.9	02.0	08.5	03.8
					100.0

TABLE 3.—Calculated numbers of fish and corresponding weights for a cohort of 1,000 albacore and estimated average annual catch in number and by age groups from 1963 through 1968 from the Bay of Biscay. See text for explanation of procedures.

Age	Cohort		Estimates for an average year's catch	
	Fish	Weight	Fish	Weight
	no.	*kg*	*no.*	*metric tons*
1	3	5.4	21,327	34.1
2	110	363.0	781,999	2,580.6
3	605	3,267.0	4,300,992	23,225.4
4	244	2,196.0	1,734,615	15,611.5
5	38	554.8	270,145	3,944.1
Total	1,000	6,386.2	7,109,078	45,394.7

Biscay from 1963 through 1968 was 45,400 metric tons (data from Food and Agriculture Organization, 1969). I assumed that the average percentages obtained for 1967 through 1970 were also representative of the years 1963 through 1968.

I used proportion to estimate the average annual catch from the Bay of Biscay in number of fish using the total weight of the cohort, the number in the cohort, and the average annual weight of the Bay of Biscay catch. This total was then separated into age-groups (Table 3) using the percentages in Table 2.

I used these figures to reconstruct three theoretical albacore populations in the North Atlantic based on total mortality coefficients of 0.50, 0.96 (determined from length frequencies), and 1.40 (Tables 4, 5, and 6). In each case I began the calculations using the estimated number of 3-year-old albacore landed. I assumed a natural mortality coefficient (M) of 0.23 and obtained the fishing mortality coefficient (F) by subtraction. I used the number of 3-year-olds landed (L_3), fishing mortality (F), and total mortality

TABLE 4.—Theoretical albacore population in the North Atlantic, ages 1 through 5, based on a total mortality coefficient (Z) of 0.50 from age 3 through 5. See text for procedures.

Age	Number of fish	Z	Total deaths	Natural deaths	Fishing deaths (surface only)
	thousands		— — *thousands of fish* — —		
1	33,372				
		0.23	6,858	6,837	21
2	26,514				
		0.27	6,273	5,491	782
3	20,241				
		0.50	7,965	3,664	4,301
4	12,276				
		0.50	4,831	3,096	1,735
5	7,445				
		0.50	2,930	--	270

TABLE 5.—Theoretical albacore population in the North Atlantic, ages 1 through 5, based on a total mortality coefficient (Z) of 0.96 from age 3 through 5. See text for procedures.

Age	Number of fish	Z	Total deaths	Natural deaths	Fishing deaths (surface only)
	thousands		— — *thousands of fish* — —		
1	15,578				
		0.23	3,201	3,180	21
2	12,377				
		0.30	3,208	2,426	782
3	9,169				
		0.96	5,659	1,358	4,301
4	3,510				
		0.96	2,166	431	1,735
5	1,344				
		0.96	830		270

TABLE 6.—Theoretical albacore population in the North Atlantic, ages 1 through 5, based on a total mortality coefficient (Z) of 1.40 from age 3 through 5. See text for procedures.

Age	Number of fish	Z	Total deaths	Natural deaths	Fishing deaths (surface only)
	thousands		— — *thousands of fish* — —		
1	11,841				
		0.23	2,433	2,412	21
2	9,408				
		0.32	2,577	1,795	782
3	6,831				
		1.40	5,146	845	4,301
4	1,685				
		1.40	1,269		1,735
5	416				
		1.40	313		270

(Z) to determine the number of 3-year olds that died (D_3):

$$D_3 = \frac{L_3 \times Z}{F} .$$

Simple back calculation using the annual mortality rates gave the original number of 3-year-olds present. I was then able to work forward and backward from this figure to obtain estimates at other ages.

Adjustments were necessary, however, in order to obtain reasonable estimates. I used much lower total mortality coefficients for 2-year-olds, for example, than for 3-year-olds. Two-year-olds were not fully recruited, and they constitute only about 10% of the total catch. I also assumed total mortality for 1-year-olds was equal to natural mortality since very few 1-year-olds are captured.

Only one of the total mortality coefficients proved to be completely unreasonable. The estimated number of 4-year-olds present was less than the estimated annual catch of 4-year-olds when $Z = 1.40$, which is obviously an impossible situation. In estimating the other two populations, $Z = 0.96$ appeared to be more reasonable than $Z = 0.50$. When the number of 4-year-old albacore that die of natural causes is obtained by subtracting the estimated fishing deaths from the estimated number of total deaths the result corresponds to an M of 0.20 when $Z = 0.96$, which is close to the assumed natural mortality coefficient of 0.23 (based on Suda's (1966) estimate) estimated for ages 1 through 3. When $Z = 0.50$, the number of natural deaths of 4-year-olds corresponds to an M of 0.32.

If we use the figures in Table 5 and apply a total mortality coefficient of 0.96 from age 5 to age 6 and 0.79 (from longline data) from age 6 through age 10, we can reproduce what theoretically occurs in the North Atlantic longline fishery in an average year. Table 7 shows that the longline fishery should capture about 718,000 albacore, ages 5 through 10, in the North Atlantic each year. The actual average number of albacore captured annually from 1963 through 1968 is estimated at 513,000 (data from Wise, 1970). This difference is large, but a relatively small adjustment in the number of recruits at age 5 would bring the figures closer together. For example, by decreasing the number of recruits to 1,100,000 the potential longline catch was calculated as 533,000 fish, much closer to the 6-year average of 513,000.

TABLE 7.—Theoretical yields from ages 5 through 10 based on the population in table 5 with natural mortality coefficient 0.23, and total mortality coefficient 0.96 from age 5 to age 6 and 0.79 from age 6 to age 10.

Age	Number of fish	Total deaths	Natural deaths	Fishing deaths
		— — — — *thousands of fish* — — — —		
5	1,344			
		829	199	360 (longline)
6	515			270 (surface)
		281	82	199
7	234			
		127	37	90
8	107			
		58	17	41
9	49			
		27	8	19
10	22			
		12	3	9
Total	2,271			718 (longline only)

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PLANKTON POPULATIONS AND UPWELLING OFF THE COAST OF PERU, JUNE 1969

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ABSTRACT

Plankton populations associated with upwelling areas and the changes with time of upwelled patches were studied off the coast of Peru near Supe in June 1969. Two patches, detected by their higher nutrient levels, greater chlorophyll pigment concentrations, and lower surface temperatures than surrounding waters, were each monitored for several days, during which time they gradually lost their identity. Actively photosynthesizing phytoplankton crops (doubling time ca. 1.4 days) of predominantly small monads and other flagellates were found in both patches. The zooplankton populations in the patch areas were estimated to be consuming no more than about 25% of the daily phytoplankton production. Direct determination of physical mechanisms affecting the patches showed a relatively high level of vertical instability in Patch 1 which would allow for turbulent mixing and the carrying of phytoplankters below the compensation depth. A horizontal divergence was associated with Patch 2 and would also have the effect of dissipating the patch. Approximate estimates of upwelling suggested vertical velocities of about 2×10^{-2} cm/sec in both patches.

Interest in biological production in Peruvian coastal waters has been high in recent years. The prosperous fish-meal industry developed around the anchovy, *Engraulis ringens*, has stimulated numerous investigations by Peruvian scientists of the food chain leading to this commercially valuable fish (see, for example: Guillén and Izaguirre de Rondan, 1968; Zuta and Guillén, 1970; and various "Informes" of the Instituto del Mar del Perú). In addition, massive upwelling and its associated biological activities along the Peru coast has been of concern to investigators from other countries and has resulted in several international cruises which have added to our knowledge of the pelagic ecology of the area.

The distribution of upwelled water at the surface is often in somewhat discrete "patches," perhaps the result of dynamic physical forces such as currents and/or eddies acting in the area. In March-April 1966, Strickland, Eppley, and Rojas de Mendiola (1969) observed low standing crops of phytoplankton (chlorophyll *a*, <2 μg/liter) in high-nutrient patches (e.g., surface NO_3-N, 20 μg at./liter or higher). In other similarly rich water phytoplankton abundance was high (chlorophyll *a*, 15 μg/liter). Barber et al. (1971) reported the surface water in an area of recent upwelling showed a lack of the "organic conditioning compounds" which may be needed for phytoplankton growth. However, in the nutrient-rich but low-chlorophyll patches examined by Strickland et al. (1969) relatively high growth rates (ca. 0.8 doubling/day averaged over the euphotic zone) were found for the phytoplankton. Similar rates (ca. 0.6 doubling/day) can be calculated from the ^{15}N (nitrate and ammonium) assimilation studies done in this area by Dugdale and co-workers (University of Washington Department of Oceanography, 1970).

A high grazing pressure exerted by the pelagic animal populations was considered by Strickland et al. (1969) as a possible underlying cause for the low standing crops in the nutrient-rich patches with active phytoplankton populations. Ryther et al. (1970) also proposed grazing as an important mechanism for the reduction of the

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phytoplankton stock in a patch of upwelled water they observed continuously for 5 days in April 1966, south of Callao. Both zooplankton and the anchovy must be considered as potentially important direct grazers of the phytoplankton. Rojas de Mendiola et al. (1969) found the stomach contents of anchovies collected in February-March of 1968 in the area of this study (i.e., off Supe) to be predominantly phytoplankton whereas those from the south (Tambo de Mora) were mainly zooplankton remains. At that time the phytoplankton crop off Supe was principally diatoms while diatoms were of much less importance in the phytoplankton populations off Tambo de Mora. Rojas de Mendiola et al. (1969) also reported they had some evidence that adult anchovies may prefer phytoplankton while animal plankters are the choice of juveniles (65-70 mm anchoveta).

In May-June of 1969 the Food Chain Research Group of the Institute of Marine Resources, University of California, in cooperation with the Instituto del Mar del Perú studied various aspects of the physical processes and biological populations associated with upwelling along the Peruvian coast during austral fall. This work was carried out as Leg 7 of the PIQUERO Cruise of the RV *Thomas Washington*. A complete description of these studies is recorded in two "unpublished" data records on file with the University of California, Institute of Marine Resources (1970,[3] 1971[4]). From these studies the coastal current system along Peru was also described (Stevenson, 1971). During PIQUERO Leg 8 the work was extended north to the Equator where the zonal circulation was studied (Stevenson and Taft, 1971). Anchovy studies (abundance, schooling, stomach contents, etc.) were carried out by Villanueva, Jordán, and Burd (1969) on board the Peruvian research vessel *SNP-1* during the first week of PIQUERO 7 in an area south of Callao.

Since neither Strickland et al. (1969) nor Ryther et al. (1970) provided quantitative evidence to support the hypothesis that grazing may be an important means of regulating the phytoplankton crop in these waters, one purpose of our present studies was to determine the abundance of the zooplankton—both microzooplankters (see Beers and Stewart, 1970) and larger forms—and to relate this to observations on phytoplankton abundance, growth rates, and taxonomic composition. In addition, we were interested in the "disappearance" of the patches with time. Evidence is examined for a suggestion that patches may dissipate through physical mechanisms such as horizontal and vertical mixing. The detailed study of physical variables has provided insight into factors affecting biological production in the waters off Peru which would not have been appreciated by the biologist working independently.

MATERIALS AND METHODS

Studies were conducted between 13 and 22 June 1969, in the approximate area bounded by lat 10° and 12° S and long 78° and 79° W off Supe. Extensive "surface" mapping operations at various times throughout this period with underway continuous analysis of inorganic nutrients, phytoplankton pigment fluorescence, and temperature allowed for the detection and surveillance of two patches of upwelled water in this area. Water for the mapping as well as for other "surface" sampling described below was taken with a pump fitted into the ship's hull about 3 m below the sea surface. This unit delivered 10 to 11 liters/min through plastic piping. A series of 57 discrete stations were made during the period of observing the patches for sampling the microzooplankton and macrozooplankton populations, obtaining water for primary production measurements and phytoplankton enumeration, and for determining temperature and salinity profiles. These were taken from various depths in and below the euphotic zone within and outside of the patches.

[3] University of California, Institute of Marine Resources. 1970. Research on the marine food chain; progress report, July 1969 - June 1970. Part III. Data record, Cruise PIQUERO, Leg 7. Section 1. Physical, chemical and production measurements off the coast of Peru, 28 May - 22 June, 1969 aboard the RV THOMAS WASHINGTON. IMR Rep. 70-5. (Unpublished manuscript.)

[4] University of California, Institute of Marine Resources. 1971. Research on the marine food chain; progress report, July 1970 - June 1971. Part III. Data record, Cruise PIQUERO, Leg 7. Section 2. The plankton. IMR Rep. 71-10. (Unpublished manuscript.)

Our purpose was to return to the same patch of water, sampling it on successive days as it "evolved." In general, the temperature and fluorescence of the surface water were used to determine the desired location of sampling after the navigational capabilities of the ship had been used to locate the general area.

Physical measurements, i.e., temperature and salinity, were either by continuous vertical profiles generally from the surface to the bottom, or 500 m, using a STD (Salinity-Temperature-Depth Measuring System, The Bissett-Berman Corp. Model 940)[5] or at the surface (3 m) using a thermo-salinograph (The Bissett-Berman Corp. Model 6600T). Both systems were electronically interfaced to a shipboard IBM 1800 computer.

Vertical profiles of current velocity were made at Stations 99 and 101 using a Hydro Products, Model 502, meter having a precision Savonius rotor to sense current speed and a direction vane coupled with a magnetic compass. The instrument, lowered on the hydrowire, measured the currents for 15 min at each of a series of depths through the upper 200 or 500 m. At each station the current direction and speed were referenced to the deepest observation. The lower practical current threshold is considered to be about 2.5 cm/sec.

Phytoplankton nutrient concentrations (i.e., PO_4, $NO_3 + NO_2$, and SiO_3) in water from the surface pump or taken by water bottles from various depths were determined using an Autoanalyzer and methods described in Strickland and Parsons (1968). A Turner fluorometer with a continuous flow-through cell (Lorenzen, 1966) was used for mapping surface phytoplankton pigment distribution. Vertical profiles of extracted chlorophyll and phaeophytin were done following the procedure of Holm-Hansen et al. (1965).

Levels of primary production through the euphotic zone were measured by the radiocarbonate uptake method at seven stations (see Table 3). Stations 59, 68, 77, and 87 were associated with Patch 1 while Stations 88, 93, and 99 were in the second mass of upwelled water (Patch 2) followed. Water was collected by Van Dorn bottles from depths corresponding to 80, 30, 20, 15, 5, and 1.5% of the surface irradiance at each location. These depths were estimated from Secchi disc depths. Incubation of samples in deck incubators cooled by surface seawater was for 6 (noon to sunset) or 24 hr.

At each site where primary production was measured, water samples were taken for analysis by the inverted microscope method of Utermöhl (1958) of the phytoplankton species composition, numerical abundance, and estimates of biomass (volume and organic carbon). Generally aliquots from the several depths sampled at each site were integrated to provide a composite sample over the euphotic zone, preserved with 5% Formalin (pH 8.2 ± 0.2), and studied as described in University of California, Institute of Marine Resources (1971, see footnote 4) and Reid, Fuglister, and Jordan (1970).

Unconcentrated samples for study of the ciliate populations were taken along with the phytoplankton as above. In addition, the ciliates of the euphotic zone were studied at eight additional sites where the larger zooplankton abundance was measured and they were also determined in integrated samples from depth intervals, generally 20 to 30 m sampled at 5-m intervals, below the photosynthetic compensation point (1.5% surface irradiance) at 10 stations.

Samples of the microzooplankton populations concentrated on 35-μ mesh cloth after excluding larger material on 202-μ mesh filters were collected from the surface (3 m) using the intake in the ship's hull at most stations where biological sampling was carried out and during certain of the mapping operations. These samples provided material for study of all microzooplankton groups other than the ciliates, many of which are too small to be retained by this size mesh. An unconcentrated pump sample taken for total ciliates is not considered here as there were indications the pump was damaging the non-loricate forms. Methods of analysis of the total microzooplankton and ciliate populations including conversion from a volume estimate to organic carbon are given in Beers and Stewart

[5] The use of trade names is merely to facilitate descriptions; no endorsement is implied.

(1970) and University of California, Institute of Marine Resources (1971, see footnote 4).

Larger zooplankton abundance was studied at 21 sites including the four stations associated with Patch 1 but only at one location, Station 93, where productivity was measured in Patch 2. Samples were taken by vertical tows from approximately 100 m to the surface using paired 0.5 m nets having 103 μ mesh. The volume of water filtered was determined by a flowmeter mounted in the mouth of one of the nets. At some stations wire angles of 10° to 25° developed during the sampling. The numerical abundance and biomass of all developmental stages of the copepod *Calanus chilensis* Brodsky were determined. An estimate of the biomass of the total Formalin-preserved net material divided into fractions of >505 μ and <505 μ was obtained as dry weight and converted to organic carbon by multiplying by 0.40. For the discussion the total material is considered to be zooplankton although there was undoubtedly a small fraction of phytoplankton and detritus associated with the net sample.

RESULTS

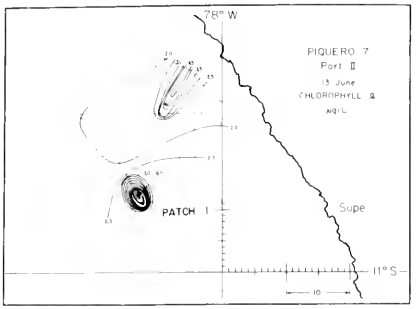
UPWELLING PATCHES

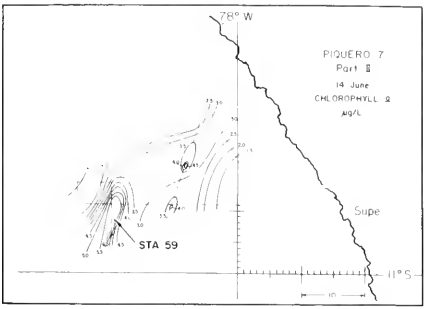
Periodic changes in position and configuration of the two patches as determined from surface mapping of phytoplankton pigments is shown in Figures 1 and 2. Patch 1, when detected on 13 June, appeared relatively compact and had a maximum surface pigment (Chl *a* and "phaeopigments") concentration of 6 to 8 μg/liter. Surrounding waters showed 1 to 2 μg/liter. The patch was bounded on the south and west by a well-defined front. Surface temperature and salinity within the patch were 19.5° C and 35.15‰, respectively, while values reached 21.0° C and 35.25‰ on moving out of the patch. The levels of temperature and salinity at the surface of the patch were similar to those found at depths of 35 to 45 m and 50 m, respectively. Surface nitrate and silicate concentrations of 16 and 13 μM, respectively, were found in the patch, dropping abruptly to about 10 and 7 μM on crossing the thermal front bordering the patch. A decline of surface pigment and nutrient levels was observed over the period 13 to 21 June. By June 17 it was apparent that this patch was disappearing and a more extensive mapping operation revealed a clearly defined patch, Patch 2, about 16 miles (25 km) south of Patch 1 (Figure 2). Patch 2 surface parameters included: maximum pigment concentration of 6 μg/liter; temperature, 19.5° C; salinity, 35.15‰; nitrate, 10 μM; and silicate, 13 μM. A distinct front bordered the patch on the west. Somewhat colder water (19.0° C) but with a lower pigment concentration ($\sim$3 μg/liter) was on the eastern side. A transect from east to west across Patch 2 passed through water with temperatures from 19° to 21.5° C. Variations of surface chlorophyll with temperature for Patch 2 are shown diagrammatically in Figure 3. This patch was no longer recognizable by 21 June. Mapping operations late in the cruise period revealed the presence of additional patches in the area but time did not allow for their study.

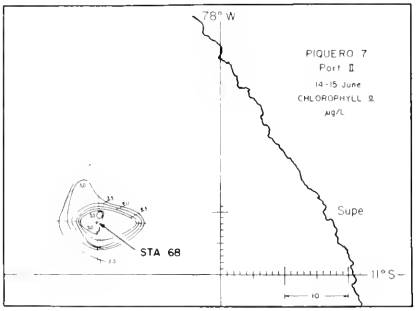
WATER MOVEMENT IN AND AROUND PATCHES

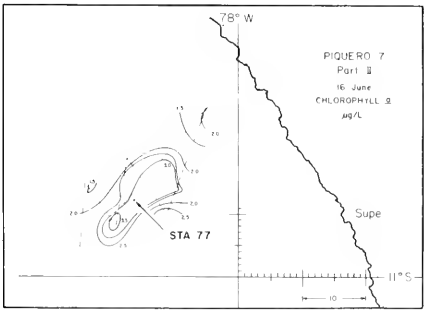
Calculations of dynamic topography suggested that Patch 1 occurred in a cyclonic eddy of the Peru Coastal Current (Figure 4). Based on the displacement of the chlorophyll pigments at the surface, the patch was being shifted to the west at about 23 cm/sec. Beneath the surface the dynamic computations suggested a poleward flow of about 15 cm/sec at 50 m (Table 1, Stations 54, 59, and 78). Cool surface water of relatively low salinity (35.15‰) was found on the nearshore side of the meander.

The limited data available for calculation of the dynamic topography around the second patch (Patch 2) suggested a northward flow of surface water along the western boundary of the front (Figure 4). Direct measurements with a current meter at Stations 99 and 101 in this patch showed a flow of 18 cm/sec to the ENE at 10 m, a stronger southerly flow of 30 cm/sec at 50 m and little current, i.e., less than 5 cm/sec, below 100 m (Table 2). Beyond the temperature-salinity front on the west of the patch, the velocity of the water was about 5 cm/sec toward the ENE









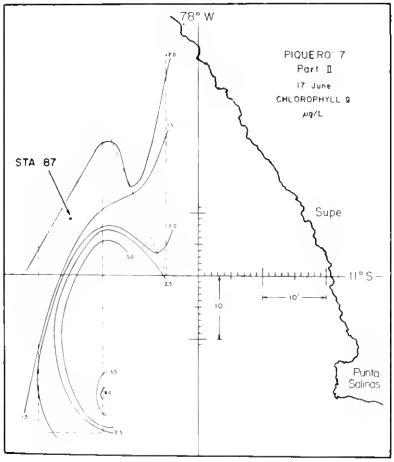


FIGURE 1.—The time sequence of changes in the configuration of Patch 1 as shown by the mapping of the total chlorophyll *a* and phaeopigments concentration (µg) liter) in the surface (i.e., 3 m) waters. In Figures 1 and 2, all chlorophyll pigment values are less than 10 µg/liter and are shown with one figure to the right of the decimal point. The decimal point is often difficult to see.

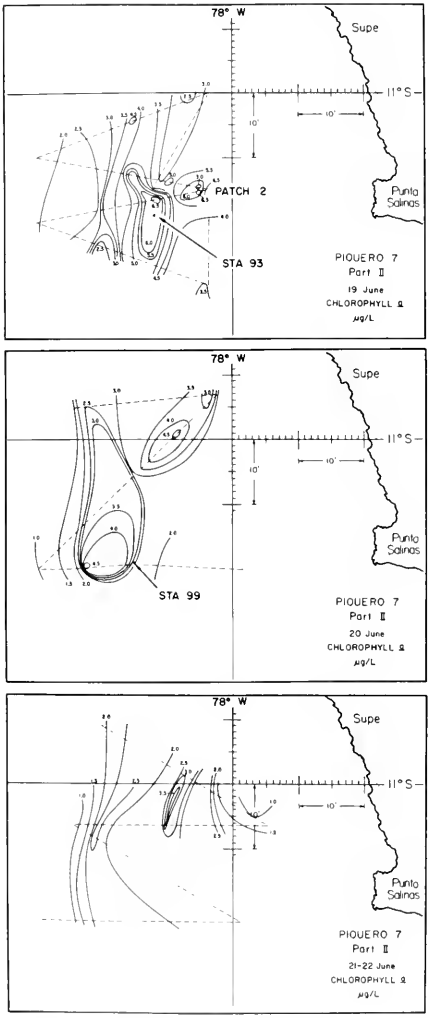


FIGURE 2.—The time sequence of changes in the configuration of Patch 2 as shown by the mapping of the total chlorophyll *a* and phaeopigments concentration (µg/liter) in the surface (i.e., 3 m) waters.

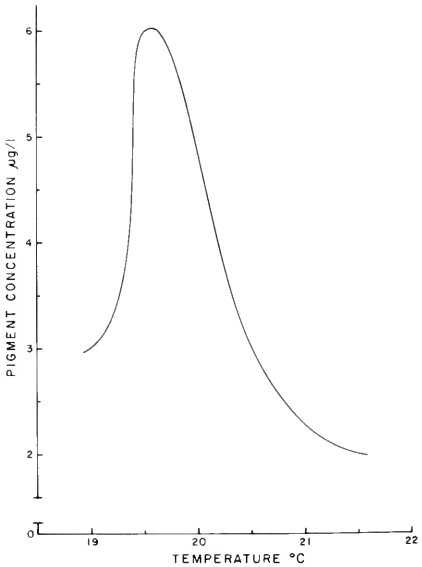


FIGURE 3.—Variation in total chlorophyll *a* and phaeopigments concentration with temperature noted in an east to west transect across Patch 2.

at 10 m (Figure 4). At greater depths at this location flow was generally eastward at velocities up to 4 cm/sec. A small northerly component was seen at 50 m. A horizontal divergence was apparent between the two stations from the zonal components of the velocity measured at 10 m.

In addition to indicating the horizontal direction of water flow, measurements from current meters can be used to estimate vertical shear (Table 2). Shear on the west side of Patch 2 was generally low because of fairly uniform flow toward the east. Maximum vertical shear inside the patch and to the east of the front was up to seven times greater than the maximum to the west of the patch. The large negative meridional (north-south) shear in the patch signified a change from weak northerly flow to strong southerly flow at 49 m.

FIGURE 4.—Dynamic topography (in dynamic meters) of the sea surface referenced to the 300 db surface. Patch 1 was observed 13-21 June 1969; Patch 2, 18-21 June 1969. Data from a detailed analysis of stations designated by a square (⊡) are given in Tables 1 and 2.

In order to obtain information on the turbulent activity of the water in the vicinity of each patch, the Väsäilä frequency, N, an indicator of static instability, and the Richardson number, Ri (Phillips, 1966), an indicator of dynamic instability, were calculated at various depths for the three stations in Patch 1 and the two current meter stations associated with Patch 2 (Table 1 and 2). The Väsäilä frequency, based on the vertical density gradient, is often used as an indication of the vertical stability in a water column assuming static conditions, i.e., no vertical shear. Small positive values for N imply the possibility of weak vertical mixing and negative values indicate the probable overturn of the water layer being studied. For the purpose of comparison it was assumed a Väsäilä number less than 1×10^{-3} sec^{-1} signified the start of static instability. Since the effects of a possible vertical shear are not considered, this measure will give an inaccurate estimate of the likelihood of significant turbulence in the presence of nonuniform horizontal currents. The Richardson number, Ri, is generally used to estimate whether turbulent mixing is an important factor for consideration. A Richardson number less than 0.25 is considered indicative of dynamic instability and the development of turbulent mixing. Owing to a lack of current meter data for Patch 1 it was necessary to estimate the shear used for determination of Ri from geostrophic currents.

In Patch 1 conditions of static instability are specifically indicated at a depth of 30 to 50 m for Station 54 (Table 1). The water columns at the other two stations associated with this patch were weakly stable. Dynamic instability was also indicated between 30 and 50 m at Station 54. In addition, the upper 10 m of Station 59 showed dynamic instability. Although static stability was greater at Station 78 than at the other two stations, the water column was dynamically unstable from the surface to 50 m because of the larger vertical shear present.

Conditions in Patch 2 (Table 2) contrasted with those in Patch 1. Vertical shear was generally much less than in Patch 1. This, however, may be partly attributable to differences arising between direct measurements of currents (Patch 2) and estimates of currents based on the horizontal distribution of mass (Patch 1). Compared with Patch 1, the water column in Patch 2 was more stable. The Richardson numbers from the west side of the front on Patch 2 are all very high and indicate conditions that are not favorable for vertical turbulent mixing. In Patch 2 the meridional component of Ri is smaller and suggests that, while the water column is not dynamically unstable in the upper 50 m, turbulent mixing might not subside as quickly as if the water column was highly stratified.

Direct current measurements to the east and west of the front (Patch 2) were made to 200 m and 500 m, respectively. If water motion within the patch at 200 m, however, was similar to the measurements at that depth on the west side of the front, the change in current velocity would be to increase the velocity in the eastward direction by about 4 cm/sec and thereby increase

TABLE 1.—Vertical shear and stability in Patch 1.

Stations	Depth (m)	Vasaila frequency, ^2N (10^{-3} sec^{-1})	Velocity, ^2v (cm sec^{-1})	Vertical sheor, $\frac{\partial v}{\partial z}$ (10^{-3} sec^{-1})	Richardson number,[3] Ri
Station 54	0		−20		
10°51.0′ S		2.5		2.0	1.53
78°13.0′ W	10		−22		
13 June 1969		4.0		3.0	1.74
	20		−25		
		2.5		−1.0	6.10
	30		−24		
		[4]--		−3.5	−1.69
	50		−17		
		12.0		−3.6	10.95
	75		− 8		
		8.3		3.6	5.34
	100		−17		
Station 59	0		18		
10°52.5′ S		2.5		7.0	0.124
78°19.6′ W	10		12		
14 June 1969		5.0		2.0	6.30
	20		10		
		14.0		3.0	21.9
	30		7		
		14.0		6.0	5.08
	50		− 6		
		8.1		8.4	0.921
	75		−28		
		8.3		−1.6	27.0
	100		−24		
Station 78	0		134		
10°54.2′ S		11.0		31.0	0.116
78°23.1′ W	10		103		
16 June 1969		10.0		29.0	0.120
	20		74		
		9.1		37.0	0.060
	30		37		
		13.0		30.0	0.205
	50		−23		
		9.8		7.2	1.85
	75		−40		
		8.3		−5.2	2.56
	100		−27		

[1] The Vasaila frequency, $N = \left(\frac{g^2\rho}{\rho^2 z} - \frac{g^2}{c^2}\right)^{1/2}$ (Phillips 1966) where g = acceleration of gravity, ρ = density of sea water, c = velocity of sound, and z = distance below sea surface.

[2] Velocities in this column represent component velocities in the NW-SE direction and are based on geostrophic computations between the individual stations and the adjacent station to the west.

[3] The Richardson number, $Ri = N^2/\left(\frac{\partial v}{\partial z}\right)^2$ (Phillips (1966) where N = Vasaila frequency and $\frac{\partial v}{\partial z}$ = vertical sheor using the component of horizontal velocity, V.

[4] The argument of the Vasaila equation was negative for this layer and signifies static instability.

the difference in zonal (east-west) velocities across the patch. Water motion in the vicinity of the patch suggests a divergent front with greater eastward water motion found on the nearshore side. The lower surface temperatures and salinity values, relative to offshore salinity, are evidence for localized upwelling and such a mechanism could provide the water needed for replacement owing to horizontal divergence near the surface.

The source for the upwelled water in the two patches we studied appears to be a poleward flow associated with a high-salinity core found at 50 m depth (Stevenson, 1971). Some of the transport from this Coastal Undercurrent is lost through upwelling as the water moves down the coastline. The undercurrent has been traced southward to lat 15°30′ S where it was still present at 50 m. The salinity in the core, however, had decreased to about 35.12‰ and the

TABLE 2.—Vertical shear and stability in Patch 2.

Stations	Depth (m)	Vaisala frequency, N (10^{-3} sec^{-1})	Velocity (cm sec^{-1}) u	Velocity (cm sec^{-1}) v	Vertical shear (10^{-3} sec^{-1}) $\frac{\partial u}{\partial z}$	Vertical shear (10^{-3} sec^{-1}) $\frac{\partial v}{\partial z}$	Richardson number[2] Ri_u	Richardson number[2] Ri_v
Station 99	10		17	5				
11°20.0′ S		12.2			−0.266	7.69	2,100	2.5
78°14.8′ W	49		18	−25				
20 June 1969		10.9			3.80	−4.20	8.2	6.7
	99		−1	−4				
		5.2			−0.101	−0.404	2,681	168
	198		[3]0	[3]0				
Station 101	10		5	1				
11°20.0′ S		14.3			1.00	−0.250	205	3,280
78°30.0′ W	50		1	2				
20 June 1969		11.8			−0.612	0.408	368	829
	99		4	0				
		5.7			0.200	0.000	822	--
	149		3	0				
		4.6			−0.204	−0.204	514	514
	198		4	1				
		4.2			0.100	0.100	1,950	1,952
	298		3	0				
		4.7			0.150	0.000	995	--
	498		[3]0	[3]0				

[1] Component velocities, u and v, are from current meter measurements and are positive to the east and north, respectively.
[2] Ri_u, Ri_v = Richardson numbers using the east and north velocity components, respectively.
[3] The deepest observations are used for reference and are shown with zero velocity.

measured velocity of about 15 cm/sec was about half that seen in this study around lat 11° S. From the PISCO Cruise of the University of Washington, April-May 1969, Smith et al. (1971) were able to estimate upwelling in a narrow coastal region near lat 15° S. They determined the vertical velocity to average 2×10^{-2} cm/sec over the period of the investigation and estimated it to decrease with increased distance from shore so as to become zero at 20 km offshore.

PHYTOPLANKTON DYNAMICS IN THE PATCHES

Photosynthetic carbon assimilation measurements (Table 3) showed that the phytoplankton crop was physiologically active in both patches throughout the periods of observation even though the patches, as defined by their surface characteristics, were gradually becoming more difficult to recognize (see Figure 5). The decline in the abundance of phytoplankters at the

TABLE 3.—Phytoplankton standing crop as carbon, photosynthetic rate, specific growth rate, chlorophyll *a* and carbon/chlorophyll *a* ratio for plankton patches off Peru, June 1969. (See Table 4 for positions of stations.)

Station	Date	Patch	Euphotic zone depth (m)	Phytoplankton standing crop (g C/m^2)	Photosynthetic rate (g C/m^2/day)	Specific growth rate (μ) (doublings/day)	Chlorophyll *a* (mg/m^2)	Ratio carbon/chl *a* (g/g)
59	14 June 1969	1	18	2.16	1.19	0.64	50.3	42.8
68	15 June 1969	1	28	1.39	0.83	0.68	45.7	30.4
77	16 June 1969	1	30	1.40	1.09	0.83	39	36
87	18 June 1969	1	42	1.91	1.01	0.61	40.8	46.7
88	18 June 1969	2	20	2.56	1.26	0.58	46.8	55
93	19 June 1969	2	27	[1]1.33	1.79	1.23	60.3	22.0
99	20 June 1969	2	30	2.27	1.03	0.54	52.9	42.9

[1] Value probably low, judged from the high μ or low C/chl *a* ratio.

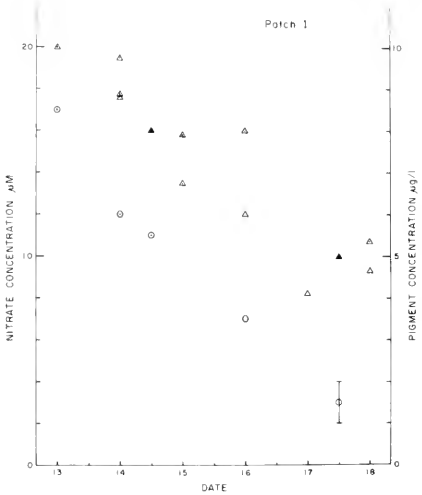


FIGURE 5.—Decline with time in surface nitrate (△) and chlorophyll pigments concentration (⊙) in Patch 1. Solid triangles (▲) are maximum nitrate values found by automatic analysis (Autoanalyzer) during underway mapping. Open triangles (△) are discrete nitrate samples collected on station. Pigment values are maxima of fluorescence found in the mapping area.

surface was compensated for by a deepening of the euphotic zone such that chlorophyll *a*, standing crop as carbon, and photosynthesis showed little variation (<twofold) when integrated over the increasing euphotic zone depth. The specific growth rate, μ, of the crop averaged 0.7 doubling/day over the euphotic zone indicating it should double in 1.4 days if grazers could be eliminated. Similar measurements of μ in March-April 1966 off Peru (Strickland et al., 1969) averaged 0.8 doubling/day for four stations.

The ratio of phytoplankton carbon/chlorophyll *a* (Table 3) averaged 40:1 and was similar to that reported by Lorenzen (1968), using different methods, for a phytoplankton bloom off Peru. The ratio of photosynthetic rate (g C m^{-2}/day) to chlorophyll *a* (g m^{-3}) averaged 28 day^{-1}, about one-half that observed by Lorenzen (1968).

As the phytoplankton crop decreased the ratio of phaeopigments to chlorophyll *a* also declined.

SPECIES COMPOSITION OF THE PHYTOPLANKTON CROPS

Taxa with volumes less than that of 10 μ spheres accounted for an average 73% (range: 46%, Station 68 to 93%, Station 59) of the total plant carbon. The dominant forms in both patches were small (2-4 μ and 5-7 μ) cells, often flagellates, and probably cryptomonads or chrysophytes although positive identification was impossible in the Formalin-preserved material. Diatoms never contributed more than 10 to 12% (average pennates + centrics, 5.2%) of the carbon content of the crop. Of the diatoms two species, *Corethron hystrix* and *Nitzschia delicatissima curva*, were the most abundant at all sites. The *Corethron* was one of the very few large taxa that would be retained by phytoplankton nets (35 μ mesh). The "photosynthetic" ciliate, *Mesodinium rubrum* (Taylor, Blackbourn, and Blackbourn, 1969), occurred at all stations but was relatively more abundant in Patch 1 where numbers reached 3000/liter at Station 68. Dinoflagellates contributed 5 to 21% of the total phytoplankton carbon. Unidentified naked forms of cell size <15 μ (equivalent sphere) were the most common of the dinoflagellates. Coccolithophorids (e.g., *Coccolithus hurleyi* and *Syracosphaera quadricornum*) were quite numerous when the patches were disappearing, totaling up to 11 to 14 μg C/liter or 31% (Station 87, Patch 1) and 15% (Station 99, Patch 2) of the plant carbon.

MICROZOOPLANKTON POPULATIONS

Ciliates dominated the microzooplankton populations of the two patches in terms of both numbers and biomass. No marked differences in the abundance of ciliates were noted between the patches. Their standing stock was greatest early in our sampling of each patch (i.e., Station 59, Patch 1; Station 88, Patch 2) owing to relatively large numbers of nonsheathed oligo-

trichs, and was correlated with the generally highest chlorophyll *a* and/or phytoplankton organic carbon level observed. The relatively small number of samples obtained, however, did not allow us to determine the variability within the patch at any given time.

Average ciliate abundance over the euphotic zone at the 15 stations studied ranged from 2400 to 18,000 liter (average, 6200 liter) (Table 4). Oligotrichous forms were at least 75% of the average numbers. Small (10-20 μ) oligotrichs without well-developed sheaths, e.g., *Lohmanniella oviformis*, were generally in much greater number than larger sheathed species, e.g., *Strombidium conicum*. Tintinnid ciliates, of which *Acanthostomella obtusa*, *Craterella urceolata*, *Codonellopsis contracta*, and *Epiplocylis brandti* were common, composed an averaged of 7% of the total numbers. Estimated total ciliate organic carbon levels (Table 4) ranged from 0.7 to 4.8 μg/liter (average, 1.9 μg/liter) of which 17% and 78%, at least, were accounted for by the tintinnids and oligotrichs, respectively.

The abundance of ciliates dropped significantly below the euphotic zone. In the approximately 20-m depth interval beneath the compensation point (10 stations observed, Table 4) ciliate organic carbon was an average 16% of that in the upper waters whereas the average chlorophyll level was still 43% of the average concentration within the euphotic zone. Ciliate abundance over the upper 100 m comparable to the water

TABLE 4.—Ciliate standing stock abundance.

Station	Date	Position	Ciliate standing stock numbers, euphotic zone (no./liter)	Ciliate standing stock biomass, euphotic zone (μg C/liter)	Ciliate standing stock biomass, below (20-m depth interval) euphotic zone (μg C/liter)
[1]59	14 June 1969	10°52.5′ S 78°19.6′ W	10,000	3.3	--
[1]77	16 June 1969	10°48.1′ S 78°16.2′ W	5,300	2.0	0.27
[1]87	18 June 1969	10°51.2′ S 78°20.4′ W	3,800	1.9	--
[1]88	18 June 1969	11°19.5′ S 78°18.6′ W	18,000	4.8	0.25
89	18 June 1969	11°19.8′ S 78°03.6′ W	5,600	1.2	0.16
[1]93	19 June 1969	11°18.8′ S 78°11.8′ W	2,900	0.9	0.38
94	19 June 1969	11°18.8′ S 78°11.8′ W	2,400	0.7	0.38
95	19 June 1969	11°20′ S 78°12′ W	3,800	1.2	0.52
96	20 June 1969	11°20′ S 78°12′ W	4,300	1.0	0.49
97	20 June 1969	11°20′ S 78°12′ W	4,000	1.3	0.43
[1]99	20 June 1969	11°20′ S 78°14.8′ W	6,500	2.0	--
100	20 June 1969	11°20′ S 78°25′ W	11,000	4.0	--
[2]102	21 June 1969	10°55.0′ S 78°24.6′ W	3,300	0.9	0.09
103	21 June 1969	10°54.2′ S 78°19.8′ W	4,700	0.9	--
104	21 June 1969	10°51.2′ S 78°20.4′ W	6,700	2.4	0.31

[1] Stations with primary productivity and phytoplankton crop taxonomic composition data.
[2] Surface chlorophyll *a* level <1.5 μg/liter.

column sampled for the "net" zooplankton was examined at Station 77. Expressed as a percentage of the average ciliate organic carbon concentration within the euphotic zone, the vertical distribution was 30 to 50 m, 14%; 55 to 75 m, 7%; and 80 to 100 m, 6%. Chlorophyll levels over the same depth intervals were 20%, 8%, and 1% of the euphotic zone average. Ciliate organic carbon estimate for the 100-m column was more than 20% of that of the total 103-μ net sample.

The numbers of small shelled sarcodinan protozoa, i.e., Foraminifera and Radiolaria, were relatively low. Numbers counted in the samples integrated over the euphotic zone were too small to provide a good estimate but suggested less than 10 foraminiferans and radiolarians/liter. At the surface, the average abundance of Sarcodina for the 12 stations was low—5.3 organisms/liter or 0.032 μg C/liter.

The metazoan microzooplankters, principally juvenile copepods, were also few in numbers. Less than one metazoan/liter was found, on the average, in +35 sample from the surface. Average numbers in the unconcentrated samples over the euphotic zone were higher (up to 40/liter) but the very few counted puts wide confidence limits on the figure. The size of the "average" individual copepod, both naupliar and post-naupliar, was significantly greater than seen previously (Beers and Stewart, 1970) and this suggests the relative absence, at least at this time of year, of the smaller species and their developmental stages compared with nutrient-rich coastal areas off California.

LARGER (+ 103 μ) ZOOPLANKTON

Standing stock zooplankton at five stations where productivity was measured (Station 59, 68, 77, 87, and 93) averaged 2.4 mgC/m^3 over the upper 100 m and showed less than a twofold difference between sites (Table 5). For the twenty-one 103-μ net samples collected, average zooplankton organic carbon was calculated to be 4.9 mg m^3. Zooplankters small enough to pass 505 μ mesh were 30% of the total at the "productivity" sites and 25% overall. *Calanus chilensis* (NVI-adults) constituted approximately

TABLE 5.—The standing stock biomass, as dry weight, of the 103 μ net zooplankton samples, 100 m to surface tows.

Station	Date	+103 μ zooplankton dry weight (mg/m^2)		
		<505 μ	>505 μ	Total
58	13-14 June 1969	228	789	1,017
59	14 June 1969	155	273	428
65	15 June 1969	420	933	1,353
66	15 June 1969	384	886	1,270
67	15 June 1969	142	147	289
68	15 June 1969	254	586	840
70	15 June 1969	594	2,410	3,004
73	15 June 1969	433	1,284	1,717
74	16 June 1969	459	1,740	2,199
75	16 June 1969	249	756	1,005
76	16 June 1969	538	1,030	1,568
77	16 June 1969	275	503	778
85	18 June 1969	399	896	1,295
86	18 June 1969	473	911	1,384
87	18 June 1969	83	351	434
93	19 June 1969	130	398	528
95	19 June 1969	145	1,169	1,314
97	20 June 1969	106	685	791

25% of total net organic carbon at the productivity sites. At other stations its estimated abundance as a percentage of the total ranged from <4% to more than 100%. It appeared as though the *Calanus* population associated with Patch 1 was staying with the patch and growing as evidenced by following a cohort of NV and NVI individuals into CI at Day 3 (June 15) and CII at Day 6 (June 18) (see University of California, Institute of Marine Resources, 1971; footnote 4).

DISCUSSION

Many of the specialized studies of biological populations and production in Peruvian coastal waters have been done during the late summer or early fall months of the southern hemisphere, i.e., February to April. The relatively large amount of data available from the Instituto del Mar del Perú provides seasonal coverage of the important food chain variables and allows us to place the conditions we found in this study during austral late fall-early winter in their proper perspective. The transport of nutrient-rich water to the surface through upwelling occurs throughout the year but it is generally most intense in winter (see Wooster and Reid, 1963; Zuta and Guillén, 1970). However, biological production, mainly as evidenced by standing crop

and stock abundance, and primary production levels (Guillén and Izaguirre de Rondan, 1968), appears to be highest during the summer period. Anchovy abundance near the coast is greatest during spring and summer, the time of the most active fishing (Sanchez, 1966).

Strickland et al. (1969) and Ryther et al. (1970) as well as others have pointed out the relatively large variation in upwelled patches at the surface. During the present cruise extensive mapping both to the north and south of Callao failed to show the very high pigment patches (chlorophyll *a*, >10 μg liter) reported by Strickland et al. (1969) in March-April 1966. Further, the two patches we followed did not have the visually brown coloration described by Strickland et al. (1969) for patches with phytoplankton in bloom conditions. The phytoplankton population of high-chlorophyll, brown-water patches has been found to be principally diatoms whereas the dominant forms in "blue" water areas were generally small flagellates (coccolithophorids and "monads"). In the regions where Rojas de Mendiola et al. (1969) reported the diet of anchovies to be phytoplankton, the crop was principally diatoms and these were the main forms, although not necessarily the same species, recognized in the gut contents of the fish. Even though the cell size of many of the diatom species reported from the brown-water patches is small they are often chain-formers which would provide a size and/or configuration that could be more easily retained on the gill rakers of the anchovy.

The size of most plant cells in the populations found during the present study is relatively small compared with the chain-forming diatoms and, therefore, these may not be efficiently utilized by the anchovy if they pass through their filtering mouthparts. If this size limitation is important, then it is plausible to suggest the anchovy will prey on larger organisms such as many of the zooplankters which in turn have grazed the small algal cells. At the time of year of this study juvenile anchovies (i.e., peladilla) of length 65 to 70 mm and of age 4 to 6 months are generally abundant, having been spawned during the spring and early summer months (see Sanchez, 1966). Rojas de Mendiola (1959)[6] and Rojas de Mendiola et al. (1969) have shown the stomach content of this size class of fish to be mainly zooplankton and have suggested a preference for this food. It would appear this could possibly be due to a lack of the diatoms or other appropriate-sized plant cells. At such times as this when the diatoms are not abundant the food chain leading to the anchovy would necessarily be lengthened by at least one extra trophic level and therefore "efficiency" of utilization of primary production in terms of production of anchovy carbon would be lessened. Growth rate (i.e., increase in length) of anchovies during these winter months is less than half that at other times of the year (Sanchez, 1966). This may be a reflection of the lower abundance of their "food" at this time of year and the need for expending a greater amount of energy to obtain the same or a lesser amount of food.

While some of the diatoms that have been reported in bloom proportions at other times of the year (e.g., Ryther et al., 1970) were present during this study, the absolute abundance of diatoms was low and they were a relatively unimportant component of the total phytoplankton crop. This could be the result of a low rate of production for these forms or may suggest they are being kept down by grazing.

Obvious possible limiting variables of the chemical and physical environment to diatom production do not seem to be relevant here. Even though these observations were made at the winter solstice, light at a location so near the Equator is probably not limiting to production, although at the time of this study the near-coastal region of Peru was often obscured by a persistent cloud cover. And, in fact, photosynthesis was saturated at an irradiance about 20% of that at the surface. Basic nutrient requirements of the diatoms should be met with the levels present. Silicate-Si at four of the five stations where productivity was measured was 8 to 10 μg at. liter or greater through the euphotic zone. Nitrate + nitrite-N levels were

[6] Rojas de Mendiola, B. 1959. Breve informe los hábitos alimenticios de la anchoveta (*Engraulis ringens* Jennys) en los años 1954-1958. A report presented to the Cía. Administradora del Guano, 30 April 1959. (Unpublished manuscript.)

generally in excess of 12 to 15 μM and PO_4-P above 1 μM. It is possible, however, that a nutrient requirement of the diatoms may not be met because of the lack of "conditioning compounds" or some similar mechanism as postulated by Barber et al. (1970). Temperature is of doubtful significance as many of the abundant species in the "blooms" enumerated by Ryther et al. (1970) and Strickland et al. (1969) might be expected to grow equally well at both winter and summer surface temperatures.

Thus, at this time of year the food chain leading to the anchovy probably consists of an intermediate zooplankton step. Villanueva et al. (1969) found stomach contents of anchovies collected 6 to 8 June during this study in an area off Punta San Juan and San Nicholas to be primarily zooplankton. Since no measure of the anchovy standing stock was made, it is not possible to estimate the predation of the anchovy on the zooplankton, but it is possible with our data to evaluate the importance of zooplankton grazing on phytoplankton as a mechanism for preventing their "blooming." The standing stock of zooplankton in the Peruvian coastal waters is generally high (Reid, 1962) although, as pointed out by Gulland (1970), there is probably a marked degree of seasonal and geographical variation in their abundance. Cushing (1969) noted an inverse correlation marked by a short lag between anchovy egg numbers and zooplankton abundance off the Peru coast during the spawning season. He implicated the spawning fish as either the direct or indirect causative agent for this. In either case it was suggested that the low zooplankton stocks and hence their reduced grazing pressure at the end of the spawning season allows for another cycle of biological production. Initially this would be of principally primary production during late summer-early fall followed by an increase in secondary production in the fall. The latter would be available to the juvenile anchovies. While this is simply speculation at the moment, hopefully it will become clearer when more data on the seasonal variation, including small-scale variations, of plankton populations are tabulated (see Gulland, 1970).

A striking feature of the zooplankton populations we observed off Peru was the great absolute and relative abundance of ciliates. The ciliates may be essential elements for the utilization of the small phytoplankton species present, and, if preyed upon in turn by the zooplankton, may represent still an additional trophic level and lengthening of the "food chain" between the primary producers and the harvestable anchovy. The average ciliate organic carbon level over the euphotic zone was about an order of magnitude greater than that found for 12 equidistantly spaced stations from lat 10° N to 12° S along long 105° W in the eastern tropical Pacific (Beers and Stewart, 1971). Ciliate abundance off Peru was similar to the average estimated for a site 1 mile off the coast of La Jolla, Calif., from weekly samples over a 5-month period in the spring and summer of 1967 (Beers and Stewart, 1970). However, off La Jolla the tintinnids accounted for almost three-quarters of the ciliate biomass. Also, just 5 to 6 miles off the California coast the average ciliate abundance (as organic carbon) over the same period had decreased to a level about one-quarter of that recorded for the present set of stations which were generally between 10 and 20 miles off the coast.

Despite their prominence in the zooplankton populations the standing stock of ciliates as organic carbon in the euphotic zone was an average of only 3.2% of the phytoplankton standing crop (6 stations with productivity data). The average daily phytoplankton production over the euphotic zone at these six sites was 49 mg C/m^3. The ciliate carbon was only approximately 5% of the new phytoplankton crop being added daily. An estimate of the fraction of the daily primary production that might be consumed by the ciliates can be made assuming the ciliates require three times their bodily carbon per day. Laboratory culture studies of pelagic ciliates (unpublished) have suggested that tintinnids may be dividing every 1 or 2 days and that the doubling time may be even shorter for the oligotrichs (see also Beers and Stewart, 1970). Values for possible ciliate consumption of new phytoplankton production ranged from 5% (Station 93) to 24% (Station 87), averaging 15%. Other microzooplankton consumption would, on the

average, be a very small addition to the total. If the ciliate populations found off Peru with their dominance of oligotrichous forms receive much of their nutritional requirements through functional chloroplasts in their endoplasm, their direct consumption of phytoplankton would probably be lower than assumed. The gymnostome ciliate, *Mesodinium rubrum*, for which good evidence of endocellular chloroplasts exists (Taylor, Blackbourn, and Blackbourn, 1969) was not included in this calculation.

The *Calanus* standing stocks at the four stations associated with Patch 1 (0-100 m) were estimated to be consuming an average of 22 mg C/m^2/day. These estimates were derived using the data of Mullin and Brooks (1970) on ingestion by the various developmental stages of *Calanus helgolandicus*. The average net primary production over the euphotic zone at these four stations was found to be 1035 mg C/m$_2$/day. Thus the *Calanus* population, which was an average of 27% of the total 103 μ net biomass was consuming only a little more than 2% of the plant production. Even if the remaining 73% of the zooplankton population were migrating to the euphotic zone and consuming phytoplankton at the same rate as *Calanus* the total consumption estimate would still be less than 10% of the daily production. Of course, a significant number of the zooplankters may not be herbivores and also many are much larger forms than *Calanus* and it is probable that their daily ingestion as a percentage of their bodily carbon would be lower than that of *Calanus*. The zooplankton populations below 100 m which might migrate vertically to feed have not been considered here. The majority of tows taken on this cruise were during daylight hours but no significantly greater abundance was evident in the few tows taken during the hours of darkness. The level of dissolved oxygen at 100 m and below in Peruvian coastal waters is low (usually <1 ml/liter). However, Mullin (1966)[7] found numerous zooplankton species inhabiting the oxygen-poor waters off Peru, and some species even showed their greatest abundance at these depths. Nevertheless, in terms of total zooplankton biomass the upper 100 m would probably be of much greater importance than lower depths.

In summary, our estimates call for no greater consumption by the zooplankton than about 25% of the daily primary production. Coupling this with the fact there was no indication that the actively photosynthesizing phytoplankton crop in either patch was increasing with time but, in fact, was actually disappearing, indicates some mechanism other than grazing must be at least partly responsible. Likewise, the fact that there was no significant increase in the phaeophytin level or in the chlorophyll phaeophytin ratio as the patch was monitored with time argues against zooplankton grazing as a principal cause. Dugdale and Goering (1970) in their study of biological production in the Peru Current during a period of high diatom levels indicated grazing was not the principal source of "loss" of phytoplankton and that the combined anchovy and zooplankton grazing was at a daily level of about 20% of the standing crop. It was further suggested that, of these, the anchovy were a quantitatively more important grazer than the zooplankters.

Strickland et al. (1969) suggested three alternate hypotheses to grazing which implicated physical factors as mechanisms for patch disappearance. In the present study, estimates of vertical shear and stability indicated that turbulent mixing was occurring in the upper 50 m in Patch 1.

Although a lack of current measurements limits our ability to accurately determine local motion within Patch 1, an order of magnitude estimate for the rate of upwelling in the patch is possible from a consideration of the size of the patch and associated biological productivity. From Figure 4 the patch size was found to be 10 km by 5 km by 50 m, in the east-west, north-south and vertical dimensions, respectively. The corresponding volume of the patch is 25×10^{14} cm^3. The patch is assumed to be 50 m thick, below which a subsurface poleward flow is

[7] Mullin, M. M. 1966. Vertical distribution of zooplankton occurring in the oxygen minimum layer off Peru. *In* University of California, Institute of Marine Resources, Research on the marine food chain, Progress report, January 1966 - December 1966, p. 359-369. (Unpublished manuscript.)

present. If the doubling rate for plankton is 1.4 days, then to maintain a constant concentration level requires that the water in the patch is removed at a rate of 2.1×10^{10} cm^3/sec. The southward transport of the undercurrent beneath the patch is estimated at 20.2×10^{10} cm^3/sec, or about 10 times the flow required for replacement of water in the patch. Upwelled water is required to replace the water being removed along the outer boundary of the patch. Since we assume that most, if not all of the water used to replace water lost from the patch, passes through the bottom of the patch at 50 m, the ascending velocity of water at 50 m is estimated to be 4.1×10^{-2} cm/sec for the patch. This is probably an upper limit since other processes also act to reduce the biomass. Unfortunately, only one sub-euphotic zone sample (30-50 m) from Patch 1 is available, and it shows a chlorophyll level only 20% of the average in the upper layer. However, if the ciliate and other zooplankton populations were grazing this material in which no new organic carbon was being produced, it would only be a relatively short time before a marked diminution of the chlorophyll level would be expected. In addition, chlorophyll levels above and below the compensation depth may not be a comparable index to phytoplankton abundance as there is an apparent decrease in chlorophyll level in many phytoplankters when kept in the dark for any period of time. Thus, the plant cell population in the sub-euphotic waters may be underestimated. The phytoplankton cells near to the compensation depth had a lower photosynthetic rate, g C/g Chl *a*/hour (University of California, Institute of Marine Resources, 1970, see footnote 3), than those higher in the water column. Eppley, Holmes, and Strickland (1967) showed that cells in such a physiological state sink at a more rapid rate than faster growing phytoplankters. While sinking or upwelling alone may not result in moving material out of the euphotic zone and preventing a "bloom" from developing, this combined with the turbulence may be a significant contributing factor. Dugdale and Goering (1970) following a high chlorophyll patch of water over 5 days, concluded that approximately 85% of the phytoplankton production at that time was being lost through sinking and mixing processes. At one site examined more closely, 16% of the standing crop was lost daily.

Continued upwelling with the consequent spreading out and or sinking of the surface waters is another mechanism which would result in masking any bloom that might have developed had the upwelled water mass remained more localized. This was suggested as a means of "preventing" blooms in regions of divergences in the Antarctic (Beklemishev, 1958). Horizontal divergence of Patch 2 with a relatively greater eastward water motion of the nearshore side was indicated by our current measurements. Water on the nearshore side would appear to "stretch" in the horizontal plane and be supplemented by ascending subsurface water. Examination of the graphic reconstruction of chlorophyll distribution (Figures 1 and 2) suggests a spreading out of the chlorophyll patches, becoming diluted in the surrounding area with time. Since dynamic vertical mixing is not indicated by the Richardson numbers for Patch 2, the decrease in chlorophyll may be largely attributed to the divergence and associated upwelling. Horizontal mixing, however, is undoubtedly an important dispersing mechanism in both patches. A rough estimate of upwelling based on the horizontal divergence was made by using the current measurements from Patch 2. Upwelling in the patch is assumed to be confined to a surface layer 50 m thick, where the vertical velocity is a maximum at 50 m (the same depth as the poleward flow of the coastal undercurrent). The resulting estimate of vertical velocity at 50 m is 2.5×10^{-2} cm/sec and compares favorably with the upwelling rate computed with greater accuracy for a nearby coastal zone (Smith et al., 1971). The surface concentration of nitrate declined in the patches over time as did the total chlorophyll pigments concentration (Patch 1, Figure 5). Nitrate consumption by the phytoplankton was calculated, assuming 1 g nitrate assimilated per 6 g of carbon fixed in photosynthesis, and the indicated plant consumption was only about one-third of the observed nitrate decline. This observation again suggests mixing of water in the patches with surrounding less rich water.

We are left with a system that is undoubtedly the result of a combination of interacting factors —both biological and physical—which can be sorted out only semiquantitatively. Apparently a loss equivalent to about 25% of the phytoplankton production may have been due to grazing by zooplankters. As measured here, diffusive mixing of the patches with adjacent waters and sinking of the phytoplankton would account for considerable additional loss of phytoplankton material. In addition other avenues of possible loss such as the direct consumption by the anchovies exist but were not evaluated here. That the relative significance of the different variables may change on a very small scale in time and location at this time of year can be suggested. Such complexity would lead to the variety of conditions that have been described from the coast of Peru.

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NOTES

OBSERVATIONS ON TWO SPECIES OF DOLPHIN (*Coryphaena*) FROM THE TROPICAL MID-ATLANTIC

Large numbers of adult dolphin, *Coryphaena hippurus* Linnaeus, aggregated at night around the U.S. Coast and Geodetic Survey Ship *Discoverer* as it drifted in the tropical mid-Atlantic Ocean in February 1969 (see Stoner, 1969). Only juveniles of *Coryphaena equiselis* Linnaeus, however, were caught under the night light. This note presents additional details of coloration and meristic counts of juvenile *C. equiselis* and reports on mid-oceanic concentrations of adult *C. hippurus*.

The drift track of the RV *Discoverer* on the Atlantic Trade Wind Expedition (ATEX)—lat 13°48′ N long 39°03′ W to lat 09°55′ N long 41° 35′ W, February 5 to 22, 1969—is shown in Figure 1 (see also Potthoff, 1969). The surface temperature of the water during the drift period ranged from 25.3° C to 26.8° C and the water depth from 1,757 fm to 2,753 fm.

Gibbs and Collette (1959) reported that very small juvenile *C. hippurus* resembled miniature feathers with dark and light bars alternating along their bodies and dorsal and anal fins. Very small *C. equiselis*, in contrast, tended to be uniformly dark along their sides, sometimes displaying weak bars along their fins. During the drift, 78 juvenile dolphin were caught by dip net; all were identified as *C. equiselis* on the basis of pigmentation on the caudal and pelvic fins (Gibbs and Collette, 1959) and vertebral counts (Collette et al., 1969). In the present sample, specimens ranging from 25 mm SL to 90 mm SL all had dark vertical bars on their bodies; the bars were most pronounced over the anal fin on the ventral half of the fish (Figure 2). The smallest *C. equiselis* juveniles (less than 25 mm SL) tended to be darker, with less pronounced

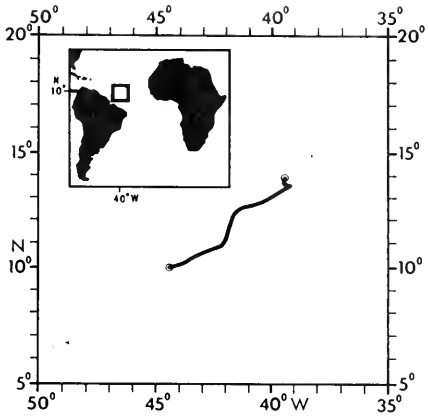

FIGURE 1.—Drift track of the RV *Discoverer* during the Atlantic Trade Wind Expedition (ATEX). The square on the insert map represents the area covered by the map.

FIGURE 2.—*Coryphaena equiselis*, 40 mm SL from the tropical mid-Atlantic Ocean, caught aboard the RV *Discoverer*, 19 February 1969.

bars or no bars on the body, and specimens larger than 90 mm SL had no bars on their bodies. A single specimen of 230 mm SL exhibited no juvenile coloration. The caudal fork margin was dark, as were the pelvic fins; however, the vertebral count (14 + 19 = 33) was that of *C. equiselis* not *C. hippurus* (Collette et al., 1969). Sixty-three of the juvenile specimens were cleared and stained to obtain vertebral counts (Table 1), leading to their positive identification as *C. equiselis*. In counting

[1] Contribution No. 194, National Marine Fisheries Service, Tropical Atlantic Biological Laboratory, Miami, Fla. 33149.

vertebrae, any vertebra associated with a pair of pleural ribs was counted as precaudal, any vertebra lacking pleural ribs was counted as caudal.

In the cleared and stained juveniles, the modal group of dorsal-fin elements was 55 to 57 (Table 1), which is more similar to the modal group of 56 to 60 for Atlantic *C. hippurus* (Gibbs and Collette, 1959) and of Pacific *C. hippurus* (Rothschild, 1964), than to that of *C. equiselis* (51-55) which these authors reported. The higher mode in these specimens of *C. equiselis* from the tropical mid-Atlantic may be representative of an oceanic population, different from that sampled by Gibbs and Collette (1959) who may have included specimens from more than one population. On the other hand, I may have counted elements in the cleared and stained specimens that were not visible to Gibbs and Collette (1959) in their untreated specimens.

Counts of the anal-fin elements of the specimens from the tropical mid-Atlantic (Table 1) were not appreciably different from those reported by Gibbs and Collette (1959). However, the mode was one fin ray higher than those from the Pacific reported by Rothschild (1964). Total gill raker counts (Table 1) of my specimens were not appreciably different from those reported by Gibbs and Collette (1959) for young *C. equiselis*, but differed from Rothschild's (1964) total counts on adult specimens by two or three rakers. My gill raker counts were made on cleared and stained juvenile specimens and did not include tooth patches on the epibranchial and hypobranchial bones; the gill raker in the epi-ceratobranchial angle was included in the ceratobranchial count. Total counts tended to decrease as fish size increased, which led me to believe that the rakers over the epibranchial and hypobranchial bones are gradually transformed into tooth patches. In specimens below 30 mm SL, the gill rakers over the epibranchial and hypobranchial bones were very small with many minute teeth. In intermediate-sized specimens (40-60 mm SL) some tooth patches could be counted over the two bones along with gill rakers. The epibranchial and hypobranchial bones of juveniles above 80 mm SL were all covered with fine teeth; the hypobranchial bone had no gill rakers associated with it, whereas the epibranchial usually had one gill raker.

Size distribution of juvenile *C. equiselis* caught during the drift period is shown in Figure 3. The mode is from 40 to 44 mm SL. From size

TABLE 1.—Frequency distribution of some meristic characters of juvenile *Coryphaena equiselis* from the tropical mid-Atlantic and data on juvenile and adult *C. equiselis* from Gibbs and Collette (1959) and Collette et al. (1969).

	Fins																												
	Dorsal fin rays																	Anal fin rays											
	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	N	$\bar{x}$	23	24	25	26	27	28	29	N	$\bar{x}$
Tropical mid-Atlantic	--	--	--	--	--	--	2	1	9	15	13	15	6	2	--	63	55.8	--	1	6	23	21	12	--	63	26.6
Gibbs and Collette (1959)	1	--	4	10	15	26	31	40	35	22	9	1	--	--	1	195	52.6	5	24	62	86	38	6	1	222	25.7

	Vertebrae						
	Precaudal		Caudal			Total	
	13	14	19	20	21	33	34
Tropical mid-Atlantic	55	8	8	55	--	63	--
Collette et al. (1969)	67	12	12	66	1	78	1

	Gill rakers											
	Epibranchial		Ceratobranchial			Hypobranchial			Total			
Tropical mid-Atlantic SL (*mm*)	1	2	9	10	11	0	1	2	11	12	13	14
<30	3	12	1	12	2	--	11	4	--	3	7	5
30-50	13	19	1	28	3	5	23	4	2	13	13	4
51-80	8	4	1	11	--	6	6	--	5	4	3	--
>80	3	--	--	1	2	2	1	--	--	3	--	--

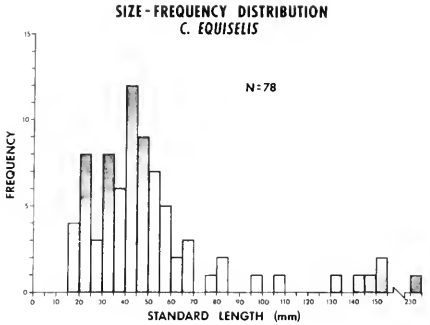

FIGURE 3.—Size-frequency distribution of *Coryphaena equiselis* which were attracted to the night light and caught in dip net during ATEX aboard the RV *Discoverer*, February 1969.

data, I infer that the species spawns in the tropical mid-Atlantic during January and February.

My specimens are presently stored at the Tropical Atlantic Biological Laboratory, Miami, Fla.

I thank Drs. Bruce B. Collette, Robert H. Gibbs, Jr., and Robert V. Miller for reviewing the manuscript, and Grady W. Reinert for preparing the illustrations.

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RESPIRATORY, BEHAVIORIAL, AND ENDOCRINE RESPONSES OF A TELEOST TO A RESTRICTED ENVIRONMENT

It is common practice when measuring fish respiration or activity to allow a varying length of time for the fish to become accustomed to the restrictions imposed by the apparatus and to allow time for any oxygen debt to be repaid (Fry, 1957). The following observations indicate that such a procedure may introduce complications in the interpretation of results since the acclimation process results in changes in respiration, behavior, and endocrine activity.

Poecilia reticulata males were placed in groups of 10 in the 100-ml chamber of a continuous flow respirometer. Animals were maintained at 25° C with a 12-hr daylength and fed daily at the start of the light period. Measurements of oxygen were made with the wide bore dropping mercury electrode (Briggs, Dyke, and Knowles, 1958) or by the micro Winkler method (Fox and Wingfield, 1938).

A daily cycle of routine respiratory activity in such an apparatus has already been described (Sage, 1968). The minimum of oxygen consumption occurs at the end of the dark period 23 hr after the last feed and this rate approximates to the standard metabolic rate. Measurement of this rate at daily intervals indicates a progressive fall in standard oxygen consumption (Table 1). A similar fall in respiratory rate

TABLE 1.—Effect of number of days in respirometer on standard rate of oxygen consumption of a group of 10 fish.

Days	Oxygen uptake $mm^3/g/hr$
1	212
2	156
3	126
4	109

to a base line of approximately 100 $mm^3/g/hr$ was observed with four other groups of fish whereas control animals from large containers

maintained a standard respiratory rate of approximately 200 $mm^3/g/hr$.

On removing these fish from the apparatus, their behavior was seen to be very different from control animals kept in 30-liter aquaria and similar to the previously described behavior of fish treated with thyroxine (Sage, 1968). Thus all fish jumped during a 15-min period after transfer from the respirometer to a 50×25 cm aquarium with a depth of 2.5 cm of water while only 17% of control animals jumped (Table 2). Similarly all fish kept for 7 days in 500-ml containers and fed ad libitum jumped when transferred to shallow water. The response was therefore to the restricted containers and not to the abnormal once-per-day feeding regime imposed in the respirometer.

TABLE 2.—Effect of maintenance conditions or thyroxine on frequency of jumping.

Treatment	Percentage jumping (number of individuals)
4 days in 100-ml respirometer	100 (30)
7 days in 500-ml container	100 (30)
28 days with thyroxine (1 in 2×10^6) in 30-liter container (Sage, 1968)	37 (28)
Control fish in 30-liter container	17 (28)

Sections through the proximal pars distalis showed a degranulation of the TSH cells in the pituitary glands of both groups of fish that had been kept in restricted environments. This was not seen in control animals. Stimulation of the thyroid gland is thus a probable cause of the observed changes in behavior and may also account for the respiratory changes. A fall in standard respiratory rate has been previously observed and attributed to progressive starvation (Fry, 1957). This cannot explain the present results since all respiratory measurements were made an equal time after a feed.

The responses reported here were obtained with container to fish volume ratios of 100 and 500:1. These are larger than the chambers used in most fish respiration studies. Thus Geyer and Mann (1939) suggested a ratio of at least 10:1 for *Perca*.

The present observations indicate that acclimating fish to a restricting apparatus may stimulate the TSH cells and thyroid and produce changes in behavior and respiration. This may be particularly confusing where seasonal changes are being investigated since thyroxine has been implicated in processes of acclimation (Hoar, 1959) and seasonal changes in fish thyroid activity are widespread (Matty, 1960; Swift, 1960) and may be related to seasonal changes in respiratory rate (Fisher, 1958).

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OCCURRENCE OF THE BARRACUDINA, *Paralepis atlantica* KRØYER, IN THE CENTRAL NORTH PACIFIC OCEAN

The barracudina, *Paralepis atlantica* Kroyer, is a bathypelagic fish that has rarely been taken by fishing gear in the North Pacific Ocean; most specimens have been recorded from waters off California in the stomach contents of predators such as whales, fur seals, and scombroid fishes (Rofen, 1966; Kajimura, 1969). Kajimura (1969) extended the known range northward on the eastern Pacific to the Washington coast (lat 46°35′ N, long 124°58′ W) with a specimen taken from the stomach of a northern fur seal, *Callorhinus ursinus*. Maruyama (1958) and Ueno and Abe (1964) reported on three specimens taken by gill net and trawl from the western Pacific Ocean near Hokkaido, Japan (between lat 42° and 43° N and near long 144° E).

The specimen reported here extends the known range of the species northward and to the central Pacific Ocean (lat 48°00′ N, long 165° 00′ W). It was captured on 6 May 1969 in surface waters of 4.5° C with a gill net fished by the RV *George B. Kelez* of the National Marine Fisheries Service. Meristic characters for the specimen are given in Table 1 with ranges for the species as reported by Rofen (1966).

The specimen was torn in half when it was removed from the gill net, preventing accurate measurements; its estimated standard length was 420 mm. It is deposited in the collection of fishes (Catalog no. 20569) at the University of Washington, Seattle.

TABLE 1.—Meristic characters of the specimen compared with those given by Rofen (1966).

Meristic characters	Specimen	Range for species
Fin rays		
Dorsal	9	9-11
Anal	23	20-26
Pectoral	16	15-17
Gill rakers		
Above angle	10	7-10
Below angle	29	25-31
Vertebrae		
Total	61	60-73
Prehaemal	35	28-38

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1) Page 202, upper left panel, in the drafting process the coordinates on the left side were not properly lined up with the computer drawn curve for the 1956 salinity; therefore, 0.25‰ should be subtracted from the 1956 salinity values obtained from the curve. The salinity curves for all other years are correct, and, of course, the harmonic coefficients for 1965 are correct.

No. 2

HUNTER, JOHN R., AND JAMES R. ZWEIFEL, "Swimming speed, tail beat frequency, tail beat amplitude, and size in jack mackerel, *Trachurus symmetricus*, and other fishes," p. 253-266.

1) Page 253, Abstract, correct equation to read:
$V - V_0 = L\ [\mathrm{K}(F - F_0)]$

2) Page 260, right column, correct equation to read:
$$\frac{V - V_0}{L} = K(F - F_0)$$

3) Page 263, Table 6, correct equation to read:
$V - V_0 = L\ [\mathrm{K}(F - F_0)]$

No. 3

HOUDE, EDWARD D., "Developmental abnormalities of the flatfish *Achirus lineatus* reared in the laboratory," p. 537-544.

1) Page 541, Figure 4 was published with the image reversed; the figure should appear: